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16. ABSTRACT

This publication represents the proceedings of two symposia conducted jointly by the US EPA and the Academy of Sciences of the USSR. The first symposia (Volume I) was held in Duluth, Minnesota, USA on October 21-23, 1975, and the second (Volume II) was held in Borok, Jaroslavl Oblast, USSR during June 22-26, 1976. The published papers from these symposia contain both broadly based review papers, designed to familiarize attendees with a wide cross-sectional representation of ecologically related activities in each country, and narrowly specific state-of-the-art scientific discussions. The presentations focus upon methodology, historical aspects, microbial and abiotic degradation processes, trace metal problems, effects of toxicants, proposed species indices, and studies of fate and transport of pollutants.

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Nitrogen	Phytoplankton	Microbiota	
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PROCEEDINGS OF THE FIRST AND SECOND USA-USSR SYMPOSIA ON THE EFFECTS OF POLLUTANTS UPON AQUATIC ECOSYSTEMS

Volume I: Duluth, Minnesota, USA Symposium
October 21-23, 1975

Volume II: Borok, Jaroslavl Oblast, USSR Symposium
June 22-26, 1976

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

ib

VOLUME I

PROCEEDINGS OF THE FIRST USA-USSR SYMPOSIUM ON THE EFFECTS OF POLLUTANTS UPON AQUATIC ECOSYSTEMS

October 21-23, 1975
Duluth, Minnesota
USA

Edited by

Donald I. Mount

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

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FOREWORD TO VOLUME I

These proceedings result from the first symposium held by Project II-1.3 of the Joint US-USSR Committee on Cooperation in the field of Environmental Protection, established in May 1972.

Broad review papers were included in the symposium in order to acquaint scientists from each country with the water pollution perspective upon which current programs are based. There are differences and therein lies the value of meeting together.

PREFACE TO VOLUME I

This volume contains nineteen of twenty papers presented at the First US-USSR Symposium on the Effects of Pollutants on Aquatic Ecosystems. All papers, ten from each side, were given in English or Russian at Duluth, Minnesota, USA between October 21 and 23, 1975, at the Environmental Research Laboratory-Duluth of the U.S. Environmental Protection Agency.

The three-day symposium climaxed a two-week visit by the Soviets as part of a working group on "Effects of Pollutants on Aquatic Ecosystems and Allowable Levels of Pollution". This is one of 40 working groups established in a five-year international "Agreement on Cooperation in the Field of Environmental Protection Between the United States of America and the Union of Soviet Socialist Republics", signed May 23, 1972 at the Moscow Summit Meeting.

During their two-week visit, Dr. Donald I. Mount, Director of the Duluth Laboratory and the U.S. Project Leader of the Working Group, brought the six visiting Soviet scientists to water pollution research laboratories in Cincinnati (Ohio), Columbia (Missouri), and Chicago (Illinois), as well as Duluth (Minnesota), to observe the American facilities and exchange technologies with U.S. researchers.

At the end of the symposium, Dr. Mount and Professor Nikolay V. Butorin, Soviet Project Leader of the Working Group, signed an agreement outlining future activities of the group, including a reciprocal visit by American scientists to the USSR in June 1976. Both countries pledged their continued commitment to cooperative environmental activities.

The publication of these proceedings is in accordance with that agreement signed October 23, 1975 by Dr. Donald I. Mount and Professor Nikolay V. Butorin.

INTRODUCTION

The Joint US-USSR Agreement on Cooperation in the Field of Environmental Protection was established in May 1972. These Proceedings result from one of the projects, Project 02.02-1.3 "Effects of Pollutants Upon Aquatic Ecosystems and Permissible Levels of Pollution".

The project derives its strength and value from the idea that it is important for scientists who share a concern for the environment to take a broad look at the subject, and to exchange views with their colleagues. It is hoped by this process to help assure that the overall goals are not lost in the clutter of minutia. These Proceedings cover Working Group II's first meeting of specialists October 21-23, 1975 at Duluth, Minnesota.

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As in any cooperative effort, many people share the success of this effort.

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SECTION 1

PERMISSIBLE POLLUTION LEVELS OF WATER BODIES

N.S. Stroganov*

The question of permissible levels of pollution for water bodies has become more and more acute because the number of substances contaminating surface waters has increased and treatment of discharges is expensive and comlex. Among engineers and other specialists who are not biologists, the concept is fairly widespread that it is possible to dump all pollutants into water because aquatic organisms will degrade them. However, at the present time such a concept is obviously erroneous and does not correspond to the actual situation. Pollution levels can vary depending on the use of the water body. Therefore, in order to talk about levels, it is necessary to establish the requirements for which the water will be used, that is, the requirements of the water users. The larger the body, the larger the number of water users will be. For complex and rational utilization of water, one must take into account the requirements of many water users. If one considers only the main requirements, meaning significance for the national economy, then one should note the following: (1) drinking and household water supply; (2) fishing industry; (3) agriculture (irrigation, livestock farms, fur farms); (4) industry (food, chemical, pulp, metallurgical, petroleum, chemical and others); (5) aesthetic and health purposes (sports, tourism, recreation, etc.); (6) transportation and certain other water uses. The quality of water can be very different for the uses mentioned. The highest water quality is needed for drinking purposes and the fishing industry, in special cases for industry (for example, the pulp industry), and the lowest quality is adequate for water transportation.

Consequently, if one satisfies the requirements for the first two water uses as to water quality, then all of the other uses will be protected. If water quality is suitable only for water transportation, then the quality of the water will be unsuitable for drinking water supplies or for the fishing industry. Therefore, in order to establish the maximum permissible level of pollution, beyond whose limits one cannot go without disrupting the use of the water, one should: first, determine the chief water users for multiple utilization of the water body, and, secondly, determine the main water quality requirements. For fresh water, almost all of the more or less large water bodies should support the interests of all water users enumerated above, and for sea water--all except for drinking purposes.

---

[1] USSR, Biology Faculty of Moscow State Univ.

As has already been noted, it is expedient to provide requirements for the first of the two water uses for fresh water and only for the second for sea water because in these cases these are the highest requirements for water quality. We will examine the level of permissible pollution taking into account these requirements and then the suitability or degree of pollution can be ascertained by comparing the existing water quality to the permissible concentrations.

Quality of water is shaped by aquatic organisms on the basis of hydrochemical and hydrological regimes. Toxicants in the water change the hydrochemical composition of the surface water and have a definite effect, depending on concentrations, on the processes determining its quality.

Pollutants discharged into surface waters gradually degrade or are transformed to less active states. The degree and rate of breakdown depend primarily on the nature of the pollutant, organisms involved in decomposition, time and physical and chemical factors (pH, O_2, salinity and hardness).

Each of the factors enumerated can accelerate or retard decomposition. Natural organic substances are fairly easily decomposed by bacteria, protozoans, fungi and other aquatic organisms. Organochlorine pesticides and detergents created by man and also heavy metals and long-lived radioactive isotopes retain their toxicity for a long time and enter into the food chain. Saprophytic and nitrifying bacteria from the Nitrosomonas and Nitrobacter groups grow and multiply poorly in the presence of toxicants; as a result, the process of recovery of the water is retarded. Figure 1 shows data on the effect of triethyl stannic chloride on biological oxidation and nitrification. Delay in decomposition of organic substances due to the effect of toxicants results in an increase (accumulation) of pollutants. If the toxicants enter waterways (rivers, canals, etc.) in significant concentrations (see Figure 1), then the water is polluted at great distances from the emission source. The nature of the pollutant and, primarily, its capability to be broken down by microorganisms will play a decisive role in the degree of pollution.

The specific composition and number of aquatic organisms play an important role in removing pollution of water. But they themselves are subject to the effect of toxic agents and therefore their biological activity and number depend on the quality and quantity of the toxicants. All of the vital processes of aquatic organisms and, consequently, the rate of detoxification of the water medium, depend on time. In the final analysis, aquatic organisms break down all toxicants or remove their toxicity, but in what time period? For us it is important now that these processes occur rapidly and completely but we can have little effect on the rate.

The physical and chemical medium has an essential meaning both for vital activity of aquatic organisms that detoxify, and for the mode of decomposition of toxicants (oxidation, ionization, hydrolysis, etc.).

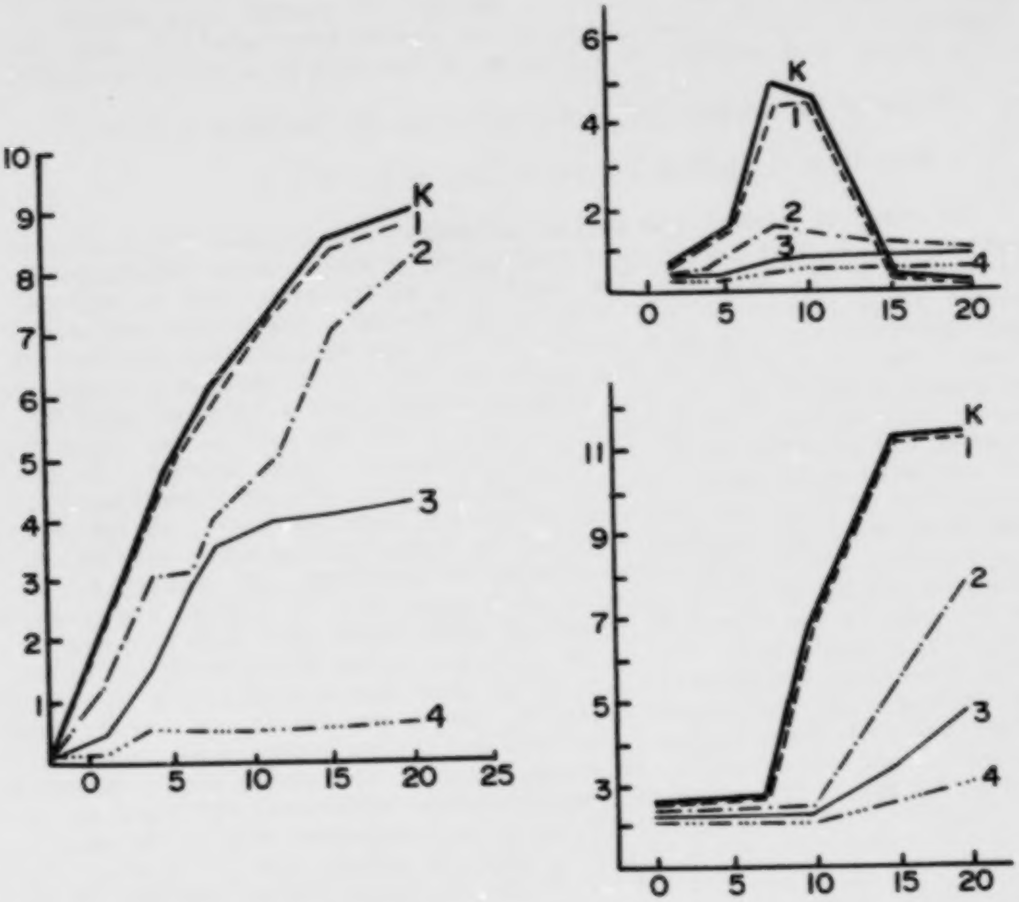

Figure 1. Processes of mineralization in solutions with triethyl stannic chloride. K--control; 1--0.1 mg/l; 2--1 mg/l; 3--10 mg/l; 4--50 mg/l. Along the abscissa--days of the experiment.

Key: a. Triethyl stannic chloride.

b. BPK [biokhimicheskoye potrebleniye kisloroda, BOD biochemical oxygen demand].

For an evaluation of a possible permissible pollution level we must make the balance between pollutants coming into a water body and the capability to degrade them completely or render them harmless. One can express these relationships in a diagram in the form of a balanced equation:

intake of pollutants (P) = decomposition (D) (self-purification)

+ deposition in bottom sediments (S), or $P = D + S$.

In order to prevent the bottom sediments from accumulating polluting substances, we must permit only that quantity which can be decomposed ($P = D$) in a unit of time. The capability of the water body to degrade wastes plays a decisive role in this equilibrium. The higher the rate of self-purification, the more pollutants that can be converted per unit of time. Ideally, for maintaining water quality, it is necessary to achieve an equality of $P = D$. In oligotrophic waters, such a balanced equality essentially exists. In eutrophic waters, $P > D$ and part of the organic substances are transferred to the bottom sediments. Cases where $P < D$ are not encountered in nature are difficult to find. The self-purification capability of water can be increased several ways, primarily by increasing the temperature and content of dissolved oxygen in the water (mixing, blowing in air), and by selecting a complex of organisms. Of course, increasing the assimilative capacity of a water body in a given time requires an increase in the number of organisms which break down such toxicants (phenols, hydrocarbons, etc.). An increase in the quantity of polluting substances over the capability to break them down may result in an increase in the number of saprophytic bacteria, fungi, protozoans and certain other organisms, but assimilation is delayed and occurs after large community reorganization. As a result, an accumulation of pollutants occurs in bottom sediments and in the water mass which creates additional difficulties in self-purification. Aquatic organisms play a decisive role in the equilibrium of water. The more intensely they can convert the pollutants, the cleaner the water will be and the larger the assimilative capacity of the water body. Biological activity of aquatic organisms, in turn, depends on living conditions. Toxicants introduced in any concentration will decrease biological activity and with high concentrations completely suppress it. Processes of growth, reproduction and effective conversion of the organism-oxidizing agents will be affected by the toxic agents. The resulting effect then will be determined by the nature of the toxicants and their concentrations. Therefore, the permissible level of pollution (PLP) is determined by the rate and degree of decomposition of the pollutant by the aquatic organisms and they determine the maximum permissible emission (MPE) of pollutants. A sequential connection of relationships reflecting a balanced equilibrium is being established. Diagrammatically it can be expressed in the following way:

complex of organisms → their biological activity → PLP → MPE.

Man is primarily interested in maximum permissible emissions. Production workers attempt to increase the size of MPE. This is economically profitable and less troublesome. However, according to the feedback principle, it destroys the structure and qualitative composition of complex organism-decomposing agents which results in reorganization of the entire community or its restructuring occurs simultaneously. Extinction, a decrease in numbers, or an increase of pollutant-tolerant organisms are the limiting conditions for uncontrolled emission of polluting substances into water bodies. While the quality of water for drinking purposes gets worse, requests to discharge more pollutants increase.

The necessity has arisen to scientifically substantiate the maximum permissible emission (MPE) of pollutants into surface waters. It seems to me that the scientific basis should be a balanced equality between the permissible level of pollution and limits on the amount of discharge.

The role of toxic agents in all of the processes of waste assimilation is tremendous because toxicants have a great effect on life processes of pollutant-decomposing organisms. Even saprophytic bacteria, as is seen in Figure 1, cannot maintain necessary biological activity in the presence of toxic agents and they themselves cannot provide initially the processes of self-purification. One should keep in mind that the nitrifying organisms are more sensitive to many toxicants than are the saprophytic bacteria. They lose, or decrease their biological activity with concentrations of certain toxicants being 10--100 times less than those affecting saprophytic bacteria (Table 1).

Substances in concentrations indicated are not completely harmless for bacteria which mineralize organic substances. The BOD and the rate of NO_2 and NO_3 formation are somewhat smaller than in the control, but decreases are less than 25% of the control. In order to achieve a control level, the processes of mineralization are increased to 3--5 days and in the presence of certain substances, a longer period is required.

Along with T.S. Balabanova we carried out tests on the breakdown of organic substances by microorganisms in a medium containing pyror-70.

A method of separate determination of BOD, NO_2 and NO_3 in closed containers was used in the first series of tests. The nutrient solution was prepared from river water, adding glucose and peptone; $(NH_4)_2SO_4$ of $NaNO_2$ were added for the nitrifying agents. They were incubated at 25 C.

In a second series of tests, open aquarium containers were used with 8 liters of solution (the same as in the first series). The quantity of organic substance was increased to a COM (chemical oxygen minimum) of 45--50 mg/liter of O_2. The addition of pyror-70 somewhat increased the COM (with 300 mg/liter pyror per 10 mg O_2/liter). Air was blown continuously through the aquarium. The temperature was 18-20 C. After a certain time samples were taken for BOD_5, NO_2 and NO_3. The results obtained are shown in the graphs in Figures 2 and 3.

Both in the closed containers (Figure 2) and in the open aquaria (Figure 3) the processes of decomposition of an organic substance were suppressed by the toxic agent--pyror-70 (2-bromo-2-nitro-1,3-propanediol); the degree of suppression was greater the higher the concentration of pyror.

Table 1. PERMISSIBLE CONCENTRATIONS (mg/l) OF TOXIC AGENTS FOR BACTERIA (saprophytic and nitrifying)

Indices	1	2	3	4	5	6	7	8
BOD	0.1	10	1	0.1	10	0.1	0.01	1.0
Formation of NO_2	1.0	1.0	0.1	0.01	0.01	0.1	0.005	0.1
Formation of NO_3	1.0	1.0	0.01	0.1	0.1	0.1	0.005	0.1

1. Aminocolophony chioroacetate
2. Pyror 400
3. Aminocolophony pentachlorophenolate (sodium salt)
4. Pentachlorophenol (precipitated $Al_2(SO_4)_3$)
5. Salicylanilide (precipitated $AlK(SO_4)_2$)
6. Salicylanilide (precipitated $ZnSO_4$)
7. Buzan-90
8. Pyror 70

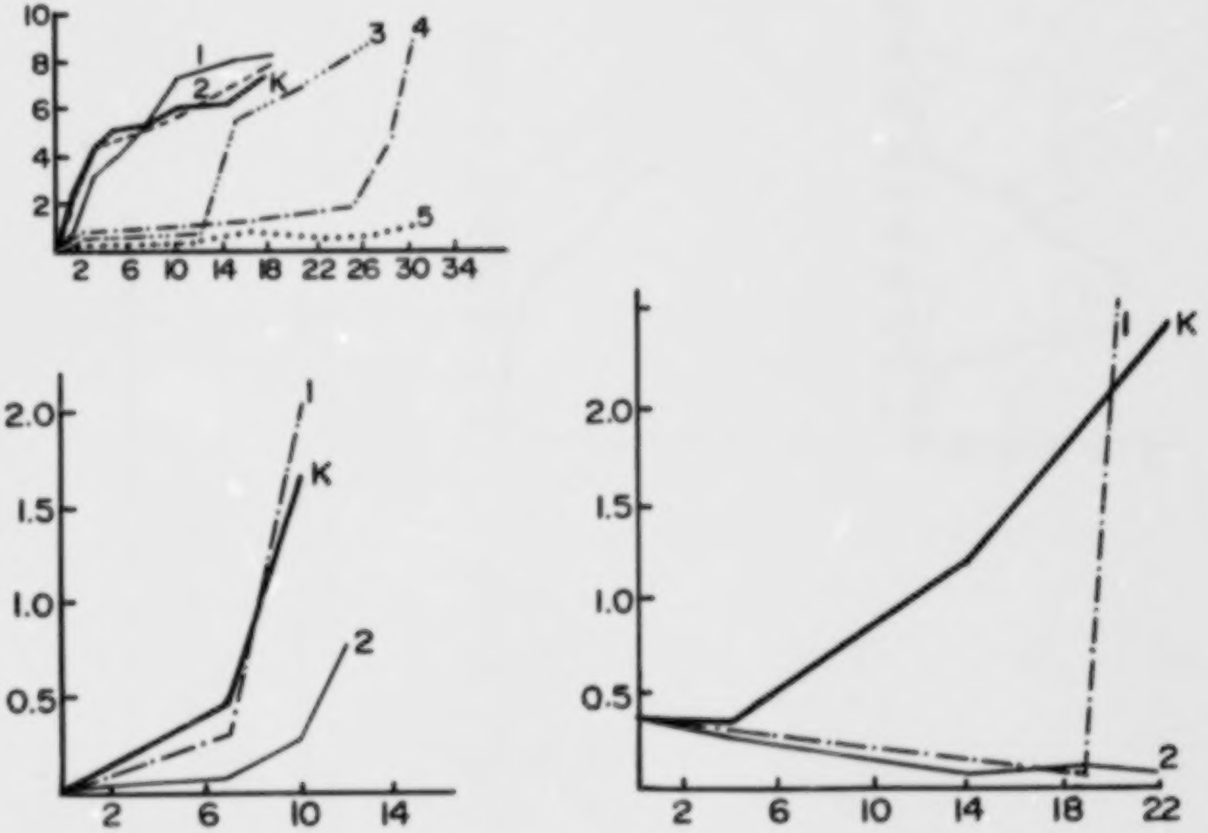

Figure 2. Processes of mineralization in solutions of pyror-70, K--control, BOD: 1--10 mg/l; 2--1 mg/l; 3--50 mg/l; 4--100 mg/l; 5--360 mg/l; 1--1 mg/l; 2--10 mg/l. Along the axis of the abscissa--days of the test.

Key: a. BOD.

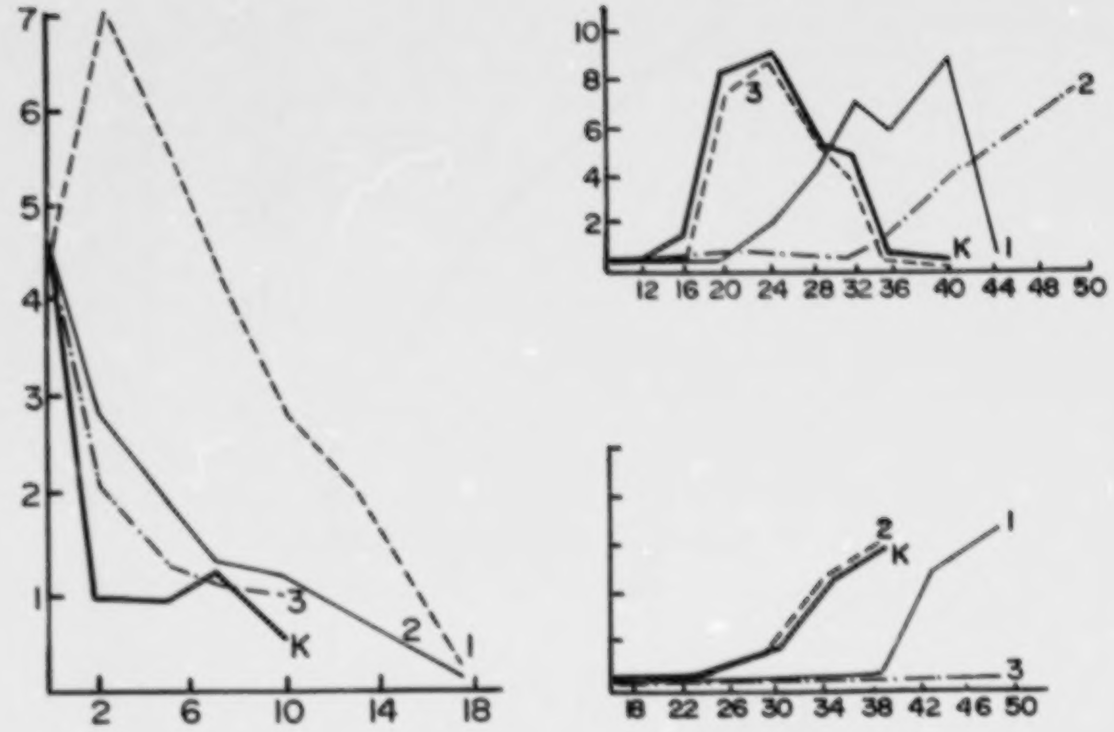

Figure 3. Processes of nitrification in solutions of pyror-70. Tests in open aquariums with air blown in. K--control.

BOD: 1--100 mg/l; 2--10 mg/l; 3--1 mg/l;
NO_2: 1--10 mg/l; 2--100 mg/l; 3--1 mg/l;
NO_3: 1--10 mg/l; 1--1 mg/l; 3--100 mg/l.

Along the axis of the abscissa--days of the test.

Key: a. BOD.

One should give special attention to the following fact. The process of decomposition of organic substances to complete mineralization occurs in the presence of a toxic agent, but to accomplish this requires a great deal of time. The shapes of the curves in Figures 2 and 3 indicates that saprophytes and nitrifying agents were hardly suppressed in their activity under the effect of pyror in the first test. The number of saprophytic organisms is changed approximately along the same curve as the BOD, NO_2 and NO_3. It seems to me that this reflects the following phenomenon. Microorganisms affected by a toxic agent die in a certain quantity. The more resistant specimens remain and, after a certain length of time and a number of generations, clones are produced which are resistant to pyror and which can carry out biological oxidation and nitrification. But, for the formation of a resistant clone, the greater the concentration of the toxic agent the more time is required.

One should note that a delay in the process of biological oxidation or nitrification (first and second phases) by 10-20 days or more is in itself an expression of pollution. If this occurs in a river in which the water is flowing at an approximate rate of 3 km/hr, then the water is not purifying (not breaking down organic substances) travels to a distance of 700-1400 km from the emission source. This situation is intensified by the fact that the processes of bacterial decomposition of organic substances occurs in a strict sequence (BOD $\rightarrow$ formation of $NO_2 \rightarrow$ formation of NO_3). Therefore, according to the balanced equilibrium, the rate of pollutant addition (P) must not be greater than the rate of decomposition (D).

In conclusion, one can formulate the following basic positions on permissible levels of pollution:

1. Different water uses permit different levels of water pollution. The lowest levels are needed for drinking water supply and fisheries.

2. Organic substances are broken down by different microorganisms in a specific sequence. Toxic substances having an injurious effect on these microorganisms suppress the processes of mineralization more strongly, the higher the concentration.

3. The maximum permissible emission (MPE) of pollutants into waters must be limited by the permissible level of pollution (PLP) of a given water at a given time. MPE, in turn, is limited by processes of self-purification (D) in which many aquatic organisms, especially microorganisms, participate. Their capability and the rate of decomposition of pollutants (D) must be appropriate to the quality and quantity of pollutants discharged.

4. Among all of the chains mentioned there must be an equilibrium of $MPE = PLP = D$. If $MPE > D$, then the water body will be polluted.

5. One cannot make calculations of values for MPE and PLP without taking into account the peculiarities of the water body, the nature of the pollutants and the season of the year.

Aquatic organisms are the main active initiators of the processes of assimilation and one must base all calculations of maximum permissible levels of pollutants on their sensitivity and capacity.

SECTION 2

A BRIEF HISTORY OF WATER POLLUTION RESEARCH IN UNITED STATES

Clarence M. Tarzwell

INTRODUCTION

Man has habitually discharged his wastes into streams. Because the United States has many large streams, large amounts of waste could be placed in them without much apparent effect. As populations in cities and industries grew, however, some streams became open sewers and people in the lower reaches began to complain. Typhoid fever became common as water supplies deteriorated. By the middle of the last century conditions had become quite bad in several areas. With little or no coordination, surveys and studies were undertaken by people in many areas. Many different approaches were used, and different studies were carried on concurrently, so it is difficult to describe the research as an ongoing program. Therefore the research for detection, evaluation, and abatement of water pollution in the United States will be described briefly under five main headings: (1) Water supply studies; (2) Pollution surveys and studies of natural purification and biological indicators of pollution; (3) Treatment of organic wastes; (4) Development, use, and standarization of bioassay methods; and (5) Determination of water quality requirements for aquatic life and development of water quality standards. In outlining these activities prime consideration will be given to the most important agencies and organizations. Early developments will be given in detail. Later work will be summarized because in recent years research has attained such diversity and magnitude that even a list of all the projects and their sponsoring organizations would be too long in a review of this type. Descriptions of developments since 1948 will be largely confined to the activities of the federal agency designated in Public Law 84-660 and subsequent federal laws dealing with water pollution.

WATER SUPPLY STUDIES

Treatment of water for domestic use may have originated in China or India thousands of years ago. In the Bible lands alum was used for the removal of turbidity as early as 900 B.C. In the fourth century before Christ Hippocrates advocated the boiling and filtering of polluted water before using it for drinking. London has been required by parliamentary statute since 1855 to filter its water supplies through slow sand filters.

Slow sand filters were first used in America in about 1870. The first important modern rapid sand filtration plant was built in 1902 at Little Falls, New Jersey.

For many years typhoid fever was a disease of prime importance. Through circumstantial evidence it was concluded that typhoid fever was usually associated with contaminated drinking water supplies. The bacterium responsible for the disease was identified in 1880. During the period 1890-1900 the incidence of typhoid fever was significantly reduced by better sanitation and filtration of water supplies. After immunization was developed in 1900, the occurrence of the disease decreased rapidly.

In the eighteen hundreds the aim was to make domestic water supplies safe. When typhoid fever was conquered, some felt the push for pollution abatement would be weakened. However, those dedicated to pollution control pointed out that the objective was to make drinking water not only safe but also palatable. Attention was then directed to tastes and odors, turbidity, and color.

The first edition of the "Microscopy of Drinking Water," by George C. Whipple, was published in 1899. This book dealt with the microscopic life other than bacteria in fresh waters. It was a compilation of limnological data and methods for the study of aquatic organisms. Although this book was concerned primarily with drinking water, it did enter the field of the natural self-purification of streams, a subject more closely associated with sewage treatment but very significant in water supply. Many investigators have studied microscopic water life, but outstanding among them are Kent, Wolle, Stokes, Zacharias, Kofoid, West, Conn, Tilden, and Calkins. In his book Professor Whipple assembled and integrated the findings of many aquatic biologists--their methods, equipment, and data. In the preface to the first edition, he mentioned especially W.T. Sedgwick of the Massachusetts Institute of Technology. He further stated, "To Prof. Sedgwick and Mr. Rafter water analysts are indebted for the most satisfactory practical method for the microscopical examination of drinking water yet devised."

It was not until the middle of the last century that the practical aspects of the study of algae and other microscopic aquatic organisms were recognized. At that time Hassall of London and Ferdinand Cohn on the Continent pointed out the correlation between microscopic aquatic life and water purity. The water works departments of the cities in the northeastern portion of the United States were the first to make studies to detect and identify filter-clogging algal blooms and growths of algae that produce tastes and odors. To the Massachusetts State Board of Health belongs the credit of having begun as early as 1887 a systematic examination of all the water supplies of the state to detect problems in their early stages so effective control methods could be initiated. In 1889 the State of Connecticut began a similar study, and city of Boston established at Chestnut Hill Reservoir a laboratory for the systematic study of the biological character of the various sources of their water supply. Algal control methods and their use developed during the first quarter of this century. In 1905 Moore and Kellerman used copper sulphate to eradicate unwanted growths of aquatic organisms. Just before the publication of Whipple's book in 1899 and in the 28 years between the first and fourth editions of this work, a great deal of effort was devoted to the study of microscopic organisms in water. Outstanding among these studies were: "A Biological Study of Lake St. Clair" in 1893 by J.E. Reighard; an examination of Lake Michigan by Henry B. Ward; and studies of the crustacea of Lake Mendota in Wisconsin by E.A. Birge. Biological stations were established by a number of midwestern universities on or in the vicinity of the Great Lakes and on the shores of smaller lakes in the Great Lakes region.

The rheological (stream) studies on the plankton of the Illinois River, begun by Kofoid in 1894 and continued through the early years of the present century as a part of the program of the Illinois Natural History Survey, have been an outstanding source of information on the influence of organic enrichment on plankton populations and the effects of these increased growths on water supplies. The investigations of the U.S. Public Health Service on the Potomac, Ohio, Illinois, Scioto, and upper Mississippi Rivers have also supplied many valuable data on organic enrichment, natural purification, and the growth of algae in streams receiving sewage and other organic wastes.

The detection and elimination of pathogenic organisms are essential for the provision of a safe drinking water supply. In their attempts to accomplish this objective, the early bacteriologists found it very difficult to detect and quantify the pathogenic organisms in water supplies. Because members of the coliform group are constantly present in alimentary discharges, their presence usually indicates fecal pollution and the possible presence of intestinal pathogens. The first test for detecting and enumerating coliforms was developed at the New York State Department of Health Laboratory in 1893 by Theobold Smith. After the further development of culture methods and procedures for enumerating them and measuring the effects of their activity, coliforms became the accepted indicator of fecal pollution. This test became the criterion and standard method for determining the sanitary quality of a water. Workers in state health departments and water pollution laboratories improved on Smith's test and devised better methods for sampling and culturing coliforms and evaluating and reporting results.

The U.S. Public Health Service also was prominent in these research efforts. After the passage in 1912 of the law authorizing the service to carry out water pollution investigations, a laboratory was established in Cincinnati, Ohio, which was known as the Stream Pollution Investigation Laboratory. In 1915 C.T. Butterfield joined the staff of this laboratory as a bacteriologist. He pioneered in the development and use of coliform tests as indicators of the sanitary quality of domestic water supplies. These tests were accepted as the tool to be used for the estimation of pollution and its natural purification, the evaluation of sewage treatment, and the sanitary quality of drinking water supplies. Butterfield was also actively engaged in the shellfish sanitation program and in the survey of the performance of representative water-treatment plants in 31 cities along the Ohio River and other rivers of the Midwest and the East. He and his small staff carried out a study of the germicidal properties of the quaternary ammonium compounds and their use and value for sanitizing milk and food utensils.

During the First World War methods were developed for the disinfection of water at army posts and for military operations in the field. An intensive and comprehensive study was made to evaluate the bactericidal efficiency of free chlorine and chloramines at different residual levels. The results of these studies were made available immediately to the army and navy, and the results guided the military in obtaining the most effective and economical use of chlorine for water disinfection. These studies established a scientific basis for municipal water-chlorination practice.

The trend of water-supply research from the 1920's into the early 1940's is indicated by the title of papers from the Cincinnati laboratory. Representative of these are the following: "The Bacteriological Examination of Water"; "The Selection of a Dilution Water for Bacteriological Examinations"; "Suggested Procedures for the Presumptive Test in the Determination of the Coli-aerogenes Group"; "Comparison of the Enumeration of Bacteria by Means of Solid and Liquid Media"; "Determining the Bacteriological Quality of Drinking Water"; "Notes on the Relation Between Coliforms and Enteric Pathogens"; "Influence of pH and Temperature on Survival of Coliforms and Enteric Pathogens When Exposed to Free Chlorine"; "Relative Resistance of *Escherichia coli* and *Eberthella typhi* to Chlorine and Chloramine"; Influence of pH and Temperature on Survival of Coliforms and Enteric Pathogens When Exposed to Chloramine"; "Bactericidal Properties of Free and Combined Available Chlorine"; Bactericidal Properties of Chloramines and Free Chlorine in Water"; and "Bactericidal Efficiency of Quaternary Ammonium Compounds."

During the 1920's and 1930's the fecal coliform tests were used in conjunction with the BOD (Biochemical oxygen demand) in all pollution surveys. Methods for conducting these tests have been included in "Standard Methods for the Examination of Water and Wastewater" by the American Public Health Association, *et al*. for many years.

At the end of the Second World War, membrane filter techniques were developed, compared with earlier procedures, and standardized. In this period studies were made to develop methods for distinguishing human coliforms from those of other animals. In the early fifties viruses in water supplies were studied at the Robert A. Taft Sanitary Engineering Center in Cincinnati. These studies were directed toward the detection, enumeration, and production of viruses in the laboratory, the determination of their effects, and their control or removal by sewage treatment. A large number of papers appeared in the 1960's describing the results of this research on viruses in water supplies.

Studies of the toxicity of heavy metals in domestic water supplies have been in progress for a number of years in several laboratories. This activity was expanded because of the increase in metals and the need for more definite data for the setting of drinking water standards. The explosive increase in the use of synthetic organic pesticides in the late 1940's and 1950's led to research programs for their detection and measurement; for the identification of their breakdown products; and for the determination of their accumulation in water, soil, and the bodies of organisms, and their metabolic pathways and passage through the food chain. Extensive studies have been and are being carried on to develop methods for collecting these materials from water supplies and other portions of man's environment and to determine their possible carcinogenic and other adverse effects.

Following the passage by the U.S. Congress in 1948 of Public Law 845, which was an important milestone in the struggle to abate pollution, the Cincinnati laboratory of the U.S. Public Health Service was enlarged and designated as the Environmental Health Center. Activities were reorganized, and more emphasis was placed on research by the establishment of a Research and Development Branch. An Aquatic Biology Section was set up under my direction. The research effort was divided between two projects: the biology of water supply and the determination of water quality criteria for aquatic life.

The water-supply unit, under the leadership of C.M. Palmer, directed its studies to the identification and control of organisms producing tastes and odors, to the identification and removal of substances producing tastes and odors, and to the identification of filter-clogging organisms and their control. The usual method for the control of tastes and odors in water supplies was to treat with chlorine or absorb the offending substances on activated carbon. The chlorine treatment was entirely experimental and often was ineffective or resulted in the production of even more odoriferous materials. The activated carbon treatment was usually successful, but it often required tremendous amounts of carbon, which were costly and presented a disposal problem. Something more exact than the cure-all chlorine treatment was needed.

The first step in meeting the problem was to grow pure cultures of those algae suspected of producing taste- and odor-causing substances to determine which species actually produced such substances. The next step was to collect and isolate those materials and determine their chemical composition. It was believed that, if the chemical compositions of these materials were known, methods could be developed for their removal from or destruction in water-treatment plants. Over 100 species of algae were grown in pure culture, but this line of research was not further supported, and equipment and staff necessary for making the chemical analyses were not secured. However, some 25 years later this same research for the determination of the composition of taste and odor materials produced by living organisms was included as a research need in the National Academy of Science, National Academy of Engineering report entitled, "Research Needs in Water Quality Criteria 1972."

Research for the development of culture methods for actinomycetes and their pure culture was also carried out for the same objective. Cultures were grown and odoriferous materials were isolated, but research for their identification was not accomplished.

The volume of sewage and other wastes discharged into our waters has increased with growth in population and the construction of sewage systems. This enrichment plus detergent carriers, certain industrial wastes, and runoff from heavily fertilized agricultural lands has produced large algal populations and the eutrophication of many lakes and reservoirs. These growths cause serious problems for water-treatment-plant operators because of the clogging of filters. In some localities at certain times backwashing requires one-fourth of the time of operation. This procedure greatly increases costs and reduces the volume of finished water produced. Known and suspected filter-clogging algae were cultured for screening tests in an effort to determine the species causing the trouble and to find a better and more selective algicide than copper sulphate.

Series of screening tests were made with new materials that were rapidly appearing on the market in the 1950's. We wanted to find algicides that were specific for the target species and nontoxic to the others. Although several good algicides were found, specific materials were not found before the research work was discontinued when biological research was transferred to the new national water quality laboratories.

In conjunction with the algicidal studies, research was carried out for the development of biological controls. We found that several algae produced materials that inhibited the growth of other algae. We also found that several algae produced antibiotics. In the course of these studies a virus that destroys some bluegreen algae was discovered. Studies of this virus have continued at the Cincinnati laboratory.

POLLUTION SURVEYS AND STUDIES OF NATURAL PURIFICATION AND BIOLOGICAL INDICATORS OF POLLUTION

The establishment of the American Fisheries Society in 1870 and the beginning of trout culture and the creation of the U.S. Commission of Fish and Fisheries in the early 1870's indicated a national awakening of interest in our fisheries and their protection. It had been noted that fishing was greatly reduced or eliminated in many streams receiving sewage or industrial wastes, or both. Fishermen began to complain and to point out the need for pollution abatement. As a result of these complaints, studies to determine the effects of pollution were undertaken.

In the 1870's Stephen A. Forbes of the Illinois State Laboratory of Natural History began investigations of the Illinois River, which later established a firm base for the comparison of stream conditions before and after pollution. The study of the Illinois River by the Illinois Natural History Survey is a classical study of the effects of stream pollution, natural purification, and biological indicators of pollution. As sewage from the city of Chicago was added to the river through the Chicago Drainage Canal, the pollution moved progressively down the river as the city and the waste load grew. This provided an excellent opportunity to observe and study the progressive chemical, physical, and biological effects of increasing pollution. Changes in color, turbidity, dissolved oxygen, carbon dioxide, bottom materials, plant growths, and aquatic animal populations were observed and recorded. The findings of these exceptionally pertinent investigations have been presented in a large number of publications appearing over a period of half a century. They describe changes in the aquatic biota as the pollution moved downstream and also the natural purification brought about by the aquatic biota found in the different areas. These studies dealt in detail with the plankton, bottom organisms, and fishes, and changes in their populations over the years as the organic load increased and the zones of pollution moved progressively downstream. Kofoid reported on "The Plankton of the Illinois River 1894 to 1899" and "Microorganisms in Reservoirs and Their Relation to Esthetic Qualities." Forbes and Richardson published a paper on "Studies on the Biology of the Upper Illinois River" in 1913 and in 1919 another paper entitled "Some Recent Changes in Illinois River Biology." In 1921 Richardson published a paper on "Changes in the Bottom and Shore Fauna of the Middle Illinois River and Its Connecting Lakes Since 1913-1915 as a Result of the Increase Southward of Sewage Pollution." In 1925 he published "Changes in the Small Bottom Fauna of Peoria Lake 1920 to 1922." These and many other reports on the effects of pollution in the Illinois River furnish valuable data on the qualitative and quantitative composition of aquatic populations in the different pollutional or life zones, their value for characterizing the extent and severity of pollution, and their role in natural purification.

After the turn of the century, many pollution surveys were made by state conservation or fish and game departments and state health departments. After the passage of the federal law of 1912, the U.S. Public Health Service made a survey of the Potomac River in 1913. At the urging of W.T. Sedgwick of the Massachusetts Institute of Technology, W.C. Purdy entered the pollution field and served as the plankton expert for the survey. He studied the biology of the river and its flats and pointed out the great value of the tidal flats for the digestion and natural purification of the organic wastes from the city of Washington, D.C. His findings were presented in a paper entitled "Investigation of the Pollution and Sanitary Condition of the Potomac Watershed."

In 1914 Purdy was transferred to the U.S. Public Health Service Stream Pollution Investigation Laboratory in Cincinnati. There he worked with the bacteriologist, C.T. Butterfield, and later with the chemist, C.C. Ruchhoft, who joined the laboratory staff in 1918. These three men and their small staff made many valuable advances in the field of water pollution research and pollution abatement. They participated in the Ohio River surveys of 1914-1918 and 1937-1941, the Illinois and Scioto River surveys, and the Lake Michigan survey. The results of their studies were reported in a series of papers under two main headings, "Experimental Studies of Natural Purification in Polluted Waters" and "Studies of Sewage Purification."

In his natural purification studies to supplement field work Purdy set up a small artificial stream using one-fourth mile of eave trough. It was built on the laboratory grounds on a slope to ensure the desired current. Water and a sewage waste were fed in at the upper end. Pollutional or life zones similar to those in sewage-polluted streams developed. This artificial stream was observed year round, and it supplied valuable data on natural purification; the role of different organisms in the purification process; seasonal changes in the pollution zones and the purification process; and the populations characteristic of the different pollutional zones.

During the period from 1914 until the Second World War, pollution surveys were made of many streams throughout the country by state and federal agencies. Several universities conducted related biological studies or co-operated with the state surveys. Birge and Juday of the University of Wisconsin made limnological studies in the lakes of the state. The New York Stream Survey under the direction of Emaline Moore (1926-1939) supplied valuable data on aquatic populations living in organically enriched streams and lakes. This survey was planned and carried out so that one or more river basins were surveyed each year. Special attention was given to polluted waters, the cause of pollution, and its effect.

In 1917 Weston and Turner at the Sanitary Research Laboratory and the Sewage Experiment Station of the Massachusetts Institute of Technology published a paper entitled, "Studies on the Digestion of a Sewage Filter Effluent by a Small and Otherwise Unpolluted Stream." This investigation was noteworthy because they studied in a natural stream the development of pollutional zones, the natural purification process, and the development of the aquatic biota characteristic of each of the zones of pollution.

As data on the extent of pollution and its effects became know, an ever-increasing demand developed from fishermen, sportsmen's clubs, civic groups, and fish and game departments for strong federal laws to control pollution. Early in the 1920's the Isaac Walton League initiated a national program for pollution abatement. This campaign was more effective than the former attempts. The passage in 1948 of Public Law 845 was due in part to the efforts of this group.

The U.S. Bureau of Fisheries and its successor, the U.S. Fish and Wildlife Service, conducted surveys in several areas. In 1927 a biological survey of the upper Mississippi River with special reference to pollution was carried out under the direction of A.H. Wiebe. Several very productive surveys were made by M.M. Ellis, Chief of the Fish and Wildlife Service field station at the University of Missouri. He approached the problem from the viewpoint of a physiologist and made many important contributions on the environmental requirements of aquatic organisms.

Ruth Patrick of the Philadelphia Academy of Natural Sciences made extensive studies of the role of plankton, especially diatoms, as indicators of stream health or pollution. In connection with these studies the "diatometer" was developed for the sampling of certain elements of the plankton population.

In 1949 the Biology Section of the Environmental Health Center in Cincinnati initiated the Lytle Creek study. This stream was selected for special study after an extensive search for a stream with one source of pollution from a sewage treatment plant and in which all the zones of pollution from recent pollution to clean water were present. The stream was surveyed, and a map was prepared showing pools, riffles and runs, and different bottom types, as well as stream widths and depths. A sampling program was established, and sampling stations were selected. A broad crested weir and gauging station were built, and a weather station was established. A trailer laboratory was equipped and placed near the Wilmington, Ohio, primary sewage treatment plant and was used as a field headquarters for chemical analyses and biological studies. Periodic sampling studies over 24-hr periods with samples taken at each sampling station every hour were conducted over a 2-year period. At least one such continuous sampling was carried out in each of the seasons for each year. During these studies hourly samples were taken for the determination of O_2, CO_2, pH, temperature, acidity, alkalinity, and turbidity. Samples were also taken for BOD and COD (chemical oxygen demand) and periodic bacteriological determinations. At selected times analyses were made for PO_4, NH_3, NO_3, and H_2S. Hourly plankton samples were taken during the 24-hr sampling periods to determine seasonal and diel fluctuations in the populations. Periodic samples of the benthic macro- and microinvertebrates were taken throughout the year under the direction of A.R. Gaufin. Monthly seinings for fish were made at all stations throughout the stream.

These studies and samplings provided data on the various pollutional zones: their biological, physical, and chemical characteristics; and seasonal and diel changes in their characteristics and extent. This extensive and intensive study produced many valuable data and several new concepts. It was concluded that the quantitative and qualitative makeup of the biota was characteristic of the so-called zones of pollution and was indicative of environmental conditions or pollution. The mere presence or absence of any single species could not be considered as an indicator of pollution. In a polluted stream O_2, CO_2, and pH could vary widely over a 24-hr period at the same station. Such variations were especially noticeable in the upper recovery zone where there were large growths of algae. These data indicated that the sag curve, developed by nonbiologists who ignored the effects of algal growths, could be very misleading, especially in the smaller streams, because the samples for its determination were usually taken after noon. Other important findings were the seasonal shift in zones of pollution and changes in their character, the extension of Sphaerotilus growth downstream in winter, the failure of fishes to enter in winter the septic zone of summer even though O_2 was abundant and the inapplicability of the K factor developed for large rivers like the Ohio River to small streams such as Lytle Creek, where it was 1.8 instead of 0.1. Data resulting from the Lytle Creek studies were reported in some 15 publications.

At the termination of the Lytle Creek studies in 1953, the laboratory bioassay studies of the Biology Section were increased. Because the water supply at the Sanitary Engineering Center was unsatisfactory for bioassay investigations and water for such studies had to be brought from the Newtown Fish Hatchery to the sixth floor of the center in glass containers, a search was made for a water supply where meaningful studies could be made. In November 1953 a cooperative laboratory was set up with the Department of Fish and Game Management of Oregon State University at Corvallis, Oregon. This laboratory was under the direction of Peter Doudoroff for the Biology Section and R.E. Dimick and C.E. Warren for Oregon State University.

Biological studies at the State of Ohio Fish Hatchery at Newtown were expanded over the years, and a temporary field laboratory was constructed in the late 1950's. Dilution water for toxicity bioassays was secured from the hatchery spring, and some of the hatchery ponds were used for field studies. Bioassay studies and the facilities at the Newtown field station were expanded under the immediate direction of Donald Mount, who joined the staff of the Biology Section in 1960. Eventually, the building was enlarged and the hatchery was secured for the toxicity bioassay studies.

TREATMENT OF ORGANIC WASTES

As communities increased in size, the need for sewage treatment and the number of sewage treatment plants increased. Investigations for the improvement of sewage treatment were carried out by state health departments and other state agencies. Rutgers University was one of the leaders in this endeavor. Valuable work was conducted by William Rudolphs and H. Heukelekian. Shortly after the establishment of the U.S. Public Health Service Stream Pollution Investigation Laboratory in Cincinnati, a series of investigations was undertaken that has continued to the present time under different names. The results of basic studies conducted during the 1910's, 1920's and 1930's were published under the general title "Studies of Sewage Purification," by Butterfield, Purdy, and Ruchhoft and their small staff. Butterfield, in cooperation with Purdy, demonstrated the role of certain protozoa in keeping bacterial populations active and efficient in the utilization and breakdown of organic materials. Butterfield investigated the die-away of coliforms in polluted waters and pioneered in isolating zoogleal bacteria from activated sludges. He also demonstrated that activated sludges consisting of pure cultures of zoogleal bacteria were capable of rapid and efficient removal of BOD from both synthetic and natural sewage.

Purdy published papers on the bulking of activated sludges as observed at the Tenafly, New Jersey, sewage treatment plant and the use of chlorine for the correction of sludge bulking in the activated sludge process. James Lackey, who worked at the Cincinnati Laboratory from the 1920's to the 1940's, published a series of papers under the general heading "Biology of Sewage Disposal." He also published numerous papers on the role of protozoa in waste treatment and water purification.

Chemical studies of the sewage-treatment and natural purification processes were conducted by Ruchhoft and his staff. They developed analytical methods for the detection and determination of waste materials and for tracing waste streams to their sources in connection with stream surveys. Considerable time was devoted to the development and improvement of the BOD and COD tests and stream-survey methods and tests. In more recent years activated carbon was used for the collection and concentration of trace materials from stream water and drinking water supplies so they could be identified and quantified. Carcinogenic substances were found to be present in small quantities in some water supplies. The carbon-filter technique for the removal and concentration of trace materials from water used for domestic supplies has in recent years brought to light the presence of undesirable substances in water supplies hundreds of miles from their point of discharge. It has also resulted in more emphasis on the detection and control of toxic and harmful materials in drinking water supplies. With the development of better analytical equipment and techniques, many problems are being detected and solved that 30 years ago were impossible to solve because of the lack of equipment and methods to detect and analyze materials occurring in very small quantities in our waters.

DEVELOPMENT, USE, AND STANDARDIZATION OF BIOASSAY METHODS

Bioassays with fish as test organisms have been used for some time to determine the acute toxicity of materials and wastes to selected test species. In 1885 McDonald reported on his studies of the toxic effects upon young shad of wastes from the Page ammoniacal works. In 1902 Knight reported on his bioassay studies, and Moore and Kellermann reported results of their bioassays in 1903 and 1905. In 1905 Marsh reported on studies of the toxicity of some industrial wastes to fish. In the same year Levy reported to the State Water Committee of Virginia on the investigation of the effects of trade wastes (sulphite waste liquor) on the waters of the James River at Richmond. In 1907 Marsh reported on the lethal dose of copper sulphate in waters of different quality. Clark and Adams reported results of their bioassay studies in Massachusetts in 1912. Wells conducted extensive bioassys, and in 1913 he reported on reactions and resistance of fishes to different concentrations of CO_2 and O_2 and in 1915 on reactions and resistance of fishes to salts in their natural environment.

The use of bioassays increased between 1910 and 1920. In 1914 Adrian Thomas conducted bioassays to test the toxicity of road tar. He used one trout fingerling in each 1500-ml container and exposed the test fish to two concentrations, 66 and 13 ppm by volume, for 3-19 days. Water in the test chambers was changed once a week or more often. Aeration was very heavy, and it may have removed some of the volatile components. In 1916 Shelford and Wells reported on the use of sunfish to determine the toxicity of gas house wastes. These were short-term acute toxicity bioassays of only 1-hr duration. An important observation was that fish do not avoid this waste, but swim into it. In 1917 Shelford reported on his continuing studies of the effects of gas house wastes on fish. In 1917 Powers described his bioassay studies in which he used the goldfish (*Carassius carassius*) as the test animal. He reported additional work in 1920 on the influence of temperature and concentration on the toxicity of salts to fishes. In 1919 Thomas of the Department of Game and Inland Fisheries of Virginia presented a paper before the American Fisheries Society on the effects of certain oils, tars, and creosotes on brook trout.

During the 1920's the use of bioassays became more widespread. The work of David Belding is noteworthy, as he had a good understanding of the factors influencing the results of bioassays and the toxicity of wastes and materials to aquatic life. In the 1924 paper by Belding, Merrill, and Kilson, "Fisheries Investigations in Massachusetts," differences in the sensitivity of different species to the same toxicant are pointed out. They found that brook trout were seven times more sensitive than carp and 28 times more sensitive than goldfish to H_2S. The authors stated, "There is a marked difference in closely allied species such as the Salmonids." They indicated that a species most sensitive to one material may be the most resistant species in the group to another toxicant. They also pointed out that fish vary seasonally in their resistance to toxicants; that the quality of the receiving water affects toxicity; and that size or weight of fish per volume of test solution, flow of water, O_2 concentration, and temperature are very important factors that influence results. Belding developed these points further in the paper he presented to the American Fisheries Society in 1927 entitled "Toxicity Experiments With Fish in Reference to Trade Waste Pollution." In this report he discussed factors that may be responsible for reported variations in toxicity of materials and wastes such as: Individual variation in resistance among members of the same species; differences in the sensitivity of a species to different toxicants; differences in the sensitivity of different species to the same toxicant; effects of age and size; differences in the dilution water, its O_2 concentration, temperature, or dissolved materials; and differences in type of test vessel used. Although he used only 24-hr tests, he recognized that longer exposures at lower concentrations would produce kills. In his bioassays he tested the toxicity of 20 materials to brook and rainbow trout, chinook salmon, carp, goldfish, and suckers.

Reports are available from several other investigators who carried out bioassays in the 1920's. In 1928 Nightingale and Loosanoff used early life stages of the chinook salmon to test the toxicity of waste sulphite liquor. Cole, Dilling, and Healey also conducted bioassays during this period. In 1924 Thomas published a paper on the absorption of metal salts by fishes. Wiebe conducted toxicity and pollution studies for a number of years and reported on exposure of young fish to varying concentrations of arsenic in 1930 and to sudden changes in pH in 1931; he also reported on effects of dissolved phosphorus and organic nitrogen in the waters of the Mississippi River in 1931.

During the 1930's bioassays were increasingly used for the evaluation of problems by state and federal investigators. Studies were made of the toxicity of cyanides, phenols, gas house wastes, pulp and paper mill wastes, oil and petroleum products, and metals. Extensive studies were also made on O_2, CO_2, temperature, and pH requirements. Many bioassay investigations were carried out by the states and the U.S. Bureau of Fisheries, which later in the decade became the U.S. Fish and Wildlife Service. Among the latter, the research of Ellis was outstanding. In 1931 Surber and Meehan reported on lethal concentrations of arsenic for certain aquatic organisms. Galtsoff made valuable contributions to knowledge of the effects of oil on marine organisms, especially its effects on shellfish. In the late 1930's and early 1940's Tennessee Valley Authority personnel engaged in extensive field investigations of the effects of malaria control--oil and paris green larviciding--on aquatic life. During the 1940's bioassays were performed at some universities. Among these the work of Anderson with Daphnia warrants special mention. Of work carried out in state laboratories that of Burdick in New York is outstanding.

With the introduction of the synthetic organic pesticides in the 1940's, there was a nation-wide surge of investigations of the toxicity of these materials to aquatic life. The U.S. Public Health Service and the U.S. Fish and Wildlife Service took dominant roles in these studies. At the Technical Development Division, Communicable Disease Center of the U.S. Public Health Service at Savannah, Georgia, I carried out and directed extensive studies of the effects of ground and airplane spraying of DDT for mosquito control on aquatic life. Effects of weekly applications of DDT and other insecticides to water areas at 0.1, 0.05, and 0.025 pound per acre were studied. Plankton, surface and benthic invertebrates, terrestrial insects (especially bees), fishes, amphibians, reptiles, birds, and mammals were studied. Applications of the insecticides were made by hand dusters and sprayers and by airplane dusts, sprays, and thermal aerosals. Results of this 3-year study were summarized in a series of papers in the Public Health Reports of the U.S. Public Health Service.

As the number of investigators performing bioassays increased, many different procedures, test organisms, dilution waters, and materials were used. This diversity resulted in great difficulty in the comparison and evaluation of the results reported by different investigators. Some uniformity in testing procedures and in the reporting of results was needed. In 1945 Hart, Doudoroff, and Greenbank published a book entitled "The Evaluation of the Toxicity of Industrial Wastes, Chemicals, and Other Substances to Fresh Water Fishes." In it they suggested procedures for care and handling of test animals, preparation of dilution water, bioassay procedures, and uniform methods for the reporting of results so that results of different investigators could be compared and the tests could be repeated. In 1949 Doudoroff, who was then on the staff of the Biology Section of the Environmental Health Center at Cincinnati, invited prominent workers in bioassay investigations to join him as members of a committee to study the various bioassay procedures being used and to select or devise and recommend procedures for bioassays which they considered best for short-term toxicity tests with fishes. Members of this committee were: P. Doudoroff, Chairman; B.G. Anderson; G.E. Burdick; P.S. Galtsoff; W.B. Hart; R. Patrick; E.R. Strong; E.W. Surber; and W.M. Van Horn. The committee met several times in Cincinnati and once in Woods Hole to draw up their recommendations. These were published in 1951 under the title "Bioassay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish." This publication and the 1945 book by Hart, Doudoroff, and Greenbank served as guides to those conducting bioassay studies and led to more uniformity in the methods used.

Doudoroff and his associate, Max Katz, published a succession of papers on bioassay studies and pertinent literature reviews while with the Biology Section during the 1950's and early 1960's. These are listed in the list of publications of the Environmental Health Center and its successor, the Robert A. Taft Sanitary Engineering Center.

During this period the U.S. Fish and Wildlife Service established a special pesticide bioassay laboratory at Columbia, Missouri, the purpose of which was to evaluate the toxicity of the new synthetic organic pesticides to aquatic life. Another laboratory was set up at La Crosse, Wisconsin, for the purpose of discovering materials or chemicals that were specific for the control of undesirable aquatic species and that would act without harm to desired organisms. This laboratory has a field station at Warm Springs, Georgia.

After 1950 the growth in the use of bioassays was so rapid and so many new workers entered the field in both the freshwater and marine environments that it is impossible to deal with all the developments and findings in a review of limited size. It is proposed, therefore, to limit the coverage of research activities after 1950 to those developments that in my opinion have been most important in leading to the present pollution abatement program of the U.S. Environmental Protection Agency.

DETERMINATION OF WATER QUALITY REQUIREMENTS FOR AQUATIC LIFE AND DEVELOPMENT OF WATER QUALITY STANDARDS

In 1950 the pollution abatement program was not progressing as expected. It appeared to me that, while some improvements had been made, overall the situation was worsening. Several approaches had been tried, but apparently a different approach was needed. Pollution abatement cases in court were drawn-out and were often lost in long arguments over what concentrations of wastes were really harmful and what really constituted pollution. Local people and the courts were influenced by threats of industry to move to another state. Some companies hired consultants to run short-term bioassays to indicate that the concentrations of their waste in the receiving water was not lethal. Hardship cases were pleaded on the grounds that industries that were forced to treat their wastes, while industries in other states were not, would be at an economic disadvantage. Further, although chemical analyses had been made and the materials in wastes identified, no firm data were available to indicate the maximum concentration of waste that was not harmful under long-term or continuous exposure. Courts were often not in sympathy with what they considered drastic action in view of the supporting data, and they and many people locally affected concluded that the only choice was fish or jobs, as suggested by industrial and chambers-of-commerce spokesmen. In such a situation they decided to take the jobs and let the environment take care of itself. Suggestions had been made that government should tell industries how to treat their wastes. Lack of such information was used by some industries as an excuse for inaction, as no one had told them how to treat their wastes at a profit. After reviewing the situation, the ever-increasing number of new wastes and materials, and the present state of knowledge as to what constituted pollution, I reached the conclusion that the best way to attain pollution abatement was to set water quality standards for each water use based on a thorough knowledge of the water quality requirements for each use. I reached this conclusion because:

1) Such standards would be uniform over large areas, everyone would be required to meet them, and no economic advantage could be acquired by anyone through exemption from treatment;

2) requirements would apply to all sections of the country, and thus there would be no incentive to move to escape them;

3) standards would be based on carefully determined requirements, and no one would be required to treat more than the essential amount; and

4) the standard, based on scientifically determined requirements, would provide a firm base for legal actions to abate pollution.

Since standards should be based on water quality requirements, the first task in a pollution abatement program is to determine water quality requirements. Because a water that is favorable for aquatic life is suitable for all other uses with recourse to available treatment methods, with the possible exception of NO_3 in drinking water and bathing waters, discussions of research in this review have been confined to those dealing with the requirements for aquatic life and water supply. Essential research that is to be considered is, therefore, that which is directed toward the determination of water quality requirements for aquatic life.

Because the determination of water quality requirements for aquatic life is largely a research problem in environmental requirements, ecology, and toxicity, a well-trained, effective, and motivated scientific staff is required along with money for the program, facilities, and equipment essential for the research. Because many of the biologists working in the U.S. Public Health Service regions felt isolated, a conference for all aquatic biologists in the Service associated with any phase of water pollution research and investigations was held in Washington, D.C. in the fall of 1950. This conference raised morale, fostered cooperation, promoted the exchange of ideas and data, and improved the research effort.

Steps were taken to acquaint the leading conservation organizations with the use and value of water quality standards in a pollution abatement program. Groups contacted were the Sport Fishing Institute, the Isaac Walton League, the National Wildlife Federation, the Wildlife Management Institute, the Audubon Society, and the National Fisheries Institute. Contacts with these groups were continued through the 1950's. I discussed the need and value of water quality standards in six papers published between 1957 and 1962.

In 1934 an annual literature review was begun by the Sewage Works Journal, now the Journal of the Water Pollution Control Federation. Over the years its coverage was widened by the inclusion of papers in the fields of stream surveys, chemistry, analytical methods, etc. A new section on the biology of water supply and water pollution was included in the review of the literature of 1953. A larger section was submitted for inclusion in the 1954 review. This dealt with bioassays, studies of the toxicity of chemicals and wastes to aquatic life, and biological indicators of pollution. The coverage was greatly expanded in the following years, and every effort was made to supply summaries of papers dealing with environmental requirements, the toxicity of wastes and other materials to aquatic life, and water quality criteria and standards.

To promote further the objectives of the meeting held in Washington, D.C., in the fall of 1950, the First Seminar on Biological Problems in Water Pollution was held in Cincinnati in April 1956. This meeting was attended by representatives from industrial concerns, academic institutions, state conservation and health departments, and federal agencies. Twenty-eight states and four provinces of Canada were represented. Biological indicators of pollution, water quality criteria, and the use and value of bioassays were discussed.

Because courts in some states were reluctant to accept as evidence the results of bioassay tests in pollution cases, it was deemed advisable to include a description of proposed bioassay methods in "Standard Methods for the Examination of Water and Wastewater" by the American Public Health Association et al. Proposed standard bioassay methods, prepared by a committee under my chairmanship and based largely on the 1951 report of the Doudoroff committee, were included in the 11th edition of this work which was published in 1960. Their inclusion was instrumental in promoting more uniform procedures, better and more comparable data, and greater use of bioassays as a research and monitoring tool for the abatement of pollution.

From 1955 through 1966 research for the determination of water quality requirements for aquatic life, the improvement of bioassay methods, and the determination of the toxicity of pesticides was promoted to the fullest extent possible by the Biology Section of the Cincinnati laboratory. The research findings of the section during this period were described in 102 publications.

The Second Seminar on Biological Problems in Water Pollution was held at Cincinnati in 1959. Attendance was much larger at this meeting than at the first seminar. The seminar theme was the effects of pesticides on aquatic life and allowable concentrations of various pesticides in the aquatic environment. Other subjects discussed were the effects of the discharge of radioactive materials, environmental requirements of aquatic life, marine and estuarine problems, and the practical application of biological findings in pollution abatement.

Contact was maintained with the private national conservation agencies, and the leaders or staff members of a number of them attended the second seminar. An advisory committee on water quality standards for aquatic life made up of the leaders of these groups was established in 1960. Members of this committee were Ira Gabrielson, director of the Wildlife Foundation

Institute; Clarence C. Cottam, director of the Welder Wildlife Foundation (formerly director and assistant director of the U.S. Fish and Wildlife Service); Richard Stroud, executive vice president of the Sport Fishing Institute; Thomas Kimball, executive director of the National Wildlife Federation; Joseph Penfold, conservation director of the Isaac Walton League; Charles Jackson of the National Fisheries Institute; and Charles H. Callison, executive vice president of the Audubon Society.

These conservation organizations presented testimony before congressional committees on various issues at frequent intervals. Some of their testimony, especially that of Richard Stroud of the Sport Fishing Institute, presented the need for and the value of national water quality laboratories, one for fresh waters and one for marine waters, to carry out research to determine water quality requirements for aquatic life. In April 1962 the House of Representatives passed legislation authorizing two water quality laboratories and appropriating money for their construction. The Senate passed a similar bill in June. The conference committees came to an agreement in August, and the bill authorizing the laboratories was signed into law on August 12, 1962.

Planning for the Third Seminar on Biological Problems in Water Pollution was under way for more than 2 years. The theme of this meeting was water quality requirements for aquatic life. Every possible effort was made to secure leading investigators to present papers and to assemble the best possible program dealing with the chosen theme. The objective was to produce a handbook summarizing available data on water quality requirements for aquatic life. Representatives of 26 nations were in attendance. Leaders of the national conservation groups took a prominent part in the seminar, which was held August 13-17, 1962, just after the passage of the legislation providing for the construction of the two water quality laboratories.

Planning for the water quality laboratories was largely completed in June 1963. Planning for the research program had been under way even before the laboratories were authorized. The first staff member for the National Water Quality Laboratory at Duluth, Minnesota, was housed in a fish hatchery of the Minnesota Department of Conservation on the shore of Lake Superior just northeast of Duluth in September 1964. The following year several thousand square feet of space was made available by the University of Minnesota at Duluth. The staff was enlarged and research activities began. Initial activities of the National Marine Water Quality Laboratory began on July 1, 1965, in office space provided by the University of Rhode Island at Kingston. A search was made for laboratory space on the coast, which could be used for research activities before the construction of the new laboratory. Since none was available, the laboratory was set up in a former industrial laboratory at West Kingston, about 8 miles from Narragansett Bay. The assembled staff moved into this building in September 1966. Laboratory furniture, equipment, and supplies, and a laboratory staff were secured and assembled for both of the water quality laboratories, and the research program for the determination of water quality requirements for aquatic life was initiated under my direction.

The bioassay laboratory that had been constructed on the grounds of the Ohio Department of Conservation hatchery at Newtown, Ohio, was made a field station of the Duluth laboratory. Construction of the National Marine Water Quality Laboratory at Narragansett, Rhode Island, was delayed, but construction of the National Water Quality Laboratory at Duluth proceeded, and it was completed in the summer of 1967 and dedicated on August 12, 1967. Construction of the National Marine Water Quality Laboratory was not initiated until August 12, 1975, 13 years after authorization.

The Federal Water Pollution Control Act, Public Law 84-660, as amended by P.L. 87-88, P.L. 89-234, and by the Clean Water Restoration Act of 1966 (P.L. 89-753), required the states to establish water quality standards for interstate waters by June 30, 1967. In case a state did not do this and failed to call a pbulic hearing, the Secretary of the Interior was authorized to set water quality standards for the interstate waters of that state. On February 27, 1967, the Secretary appointed an advisory committee to recommend water quality criteria for the following uses: Aesthetics and recreation; public water supply; fish, other aquatic life, and wildlife; and agricultural and industrial water supplies.

The committee on fish, other aquatic life, and wildlife was composed of 28 members of varied training and experience who collectively covered all phases of the subject and represented a great deal of experience in bioassay studies and water quality requirements for aquatic life. Their task was to review available data on the water quality requirements of aquatic life and then, on the basis of available data, their experience, and judgement, to recommend water quality criteria. Their report was completed by mid-June 1967. Their report on research needs was completed in the spring of 1968, and both reports were published in April 1968 along with the reports of the other committees. This report was updated and expanded by a large committee of the National Academy of Sciences and the National Academy of Engineering and was published in 1974 under the title "Water Quality Criteria 1972."

The compilation of data for the 1968 report demonstrated that practically all the bioassay studies were of short duration and indicated only the acute effects of toxicants on fishes. Methods had been suggested for the use of application factors with data from acute toxicity studies to predict long-term effects of toxicants, but few data were available to indicate the maximum concentration of a toxicant in the aquatic environment that was not harmful with continuous exposure. Studies of physical environmental requirements, especially temperature and O_2, has received the most attention, and several field studies that extended over longer periods had been made. Oxygen and temperature requirements of fishes were investigated by a number of workers in the 1920's and 1930's. However, most of the investigators reported on temperature and oxygen levels that were lethal, and very few dealt with conditions that were favorable for the survival of the species or that enabled them to compete successfully with their competitors and predators. In the late 1920's Belding gave a good analysis of the problem. There was no overall planning or coordination of the investigations of environmental requirements, which were carried on by investigators of diverse training, experience, and interests who were scattered throughout the country. The best work on environmental requirements in this period was that of M.M. Ellis and his staff. His paper on "Detection and Measurement of Stream Pollution" has become a classic. His recommendation of a minimum of 5 mg/liter of O_2 for a well-rounded fish population is still being used. It is good because it is based on field studies in a large number of streams. Mention should be made of the research carried on during the 1940's and 1950's at the University of Toronto and its field station by F.E.J. Fry. His work and that of his students had a great deal of influence on research on environmental requirements. His leadership and foresight made his laboratory a world leader in temperature and oxygen-requirement studies. In the mid-1950's a long-term study of the oxygen requirements of fishes was initiated at the cooperative water pollution laboratory of the Biology Section of the Robert A. Taft Sanitary Engineering Center and Oregon State University under the direction of Doudoroff and Warren.

The passage of the Water Quality Act of 1965 and the Clean Water Act of 1966 requiring the states to establish water quality standards stimulated country-wide research on water quality requirements. This legislation and increased public awareness of the ever-increasing detrimental effects of water pollution due to population increases, industrial development, and new highly toxic products caused an increase of research efforts in freshwater, estuarine, and marine environments. The growth in size and number of thermal electric generating plants and the construction of nuclear plants caused a phenomenal increase in studies on the effects of temperature on the aquatic environment. The great increase in sea transport of oil and the Torrey Canyon spill brought a similar increase in studies of the effects of petroleum products on the aquatic environment. In addition, for about 30 years the toxicity of pesticides (insecticides, herbicides, algicides, fungicides, etc.) and their effects on aquatic and terrestrial non-target species including man had been an ongoing problem. Water pollution hearings and enforcement actions requiring hard evidence brought the water quality researchers to the front lines for the presentation of data, the collection of evidence, and the recommendation of criteria and standards.

The increased and broadened research due to the above factors produced a tremendous increase in the use of bioassays. With the expansion of the investigations into the marine environment, there was a great increase in the use of different groups and species as test organisms, creating a need for additional bioassay methods. In 1966 the standard bioassay methods committee began to prepare materials for the 13th edition of "Standard Methods for the Examination of Water and Wastewater." Subcommittees were set up in each of the water quality laboratories and prominent investigators in other federal agencies and the states were invited to serve on the committee. Although I, as chairman, wished to include new methods for marine organisms, the committee felt the tests were not yet well developed, and the only new material in the 13th edition, printed in 1971, dealt in the long-term tests for fishes.

Although we have many marine laboratories in the United States, only a few conducted research on water pollution problems until fairly recently. Some of the early studies were carried out by Julius Nelson, who began his investigations of oysters in the last quarter of the past century. His son, Thurlow Nelson, continued the studies on oysters and presented his findings in several reports from the Department of Biology of Rutgers University (1917, 1921, 1923, 1927, 1936, and 1938). In the 1950's bacteriological studies were made on sewage disposed of through ocean outfalls by the determination of coliforms and other bacteria, their abundance and rate of decrease, and the factors affecting each. Studies were also made to evaluate bacterial contamination, bacteria in sediments, bacteria in submerged outfalls, the bactericidal action of sea water, and the survival of enteric organisms in sea water.

During the past 20 years biological oceanography and marine biology have recieved more attention. Stations are now established at the mouths of estuaries, and the emphasis has changed from observational, physical, chemical, and taxonomic studies to biological, physiological, environmental, and ecological approaches including bioassays, toxicological studies, environmental requirements, and mariculture. Mariculture, or the rearing of food organisms, is important especially in that it provides techniques for the rearing, care, and handling of test organisms for bioassays and the production of different life stages of organisms in sufficient numbers for bioassay tests. The development of methods for rearing mrine organisms is basic for long-term bioassays extending through a portion or the entire life cycle of an organism.

From November 1972 through April 1974, I devoted a portion of my time to a survey of the marine laboratories of the Atlantic and Gulf coasts. This provided first-hand information on the various research projects under way on the toxicity of pesticides, metals, and other materials; on the environmental requirements of estuarine organisms; and on the culture not only of fishes, but also of many other marine organisms important commercially and as food for other organisms. Some of the subjects that are now being or have recently been studied are: (1) Microbial decomposition of oil and pulp mill wastes; (2) bioaccumulation of heavy metals by littoral and pelagic marine animals; (3) effects of toxicants on the larval stages, juveniles, and adults of marine animals to ascertain the most sensitive stages in the life cycle; (4) potential environmental disturbances due to marine mining operations as a basis for developing appropriate marine mining techniques; (5) decay of pesticides in marine sediments, its rates and pathways, and identification of decomposition products and their effects; (6) the distribution of radionuclides in the marine environment; and (7) accumulation of persistent organic compounds in phytoplankton and their effects. In addition, studies are being made of the effects of ocean dumping of wastes; the collection of potential toxicants in bottom deposits; the return of toxicants from benthic sediments; the increase of dissolved toxicants in marine water; the bioaccumulation of toxicants; the fate and effects of oil, pesticides, and metals in the marine environment; and the development of bioassay methods for use with marine invertebrates.

Planning for the bioassay section of the 14th edition of "Standard Methods for the Examination of Water and Wastewater" began in 1971. To secure the broad coverage needed to meet the problems facing investigators and furnish methods for bioassays with the broad spectrum of organisms being used or which should be used in the toxicological and water quality studies, subcommittee chairmen were appointed for each of the main groups of organisms commonly used in bioassays--i.e., phytoplankton, zooplankton (protozoa, copepods, and *Daphnia*), corals, worms, crustaceans, aquatic insects, mollusks, and fishes. Although several of the suggested methods had not been extensively tested, it was considered imperative to make a beginning so much-needed methods for these groups could be used and improved. The 14th edition of this work was published in 1975. It is hoped that these suggested methods will provide the base upon which adequate bioassay methods for all the different groups of organisms can be developed to meet the needs for the determination of water quality requirements, the detection and evaluation of pollution, and the supplying of data necessary for setting effluent requirements, granting discharge permits, and enforcement actions.

The passage of Public Law 92-500 in 1972 added another great need and impetus for research to supply the required information for pollution abatement. Research for the determination of water quality requirements on which water quality standards must be based is now developing and expanding rapidly, and more of it is now in the right direction. I have had the privilege of knowing the men who pioneered water pollution research from 1910 to 1930 and discussing problems with them. These contacts and my field and laboratory investigations in aquatic biology, ecology, environmental improvement, fisheries management, pollution abatement, and water quality requirements since 1928 have been a wonderful experience. Although much remains to be done and there have been mistakes and defeats, good progress is now being made. With qualified and motivated leaders, well trained and experienced in the work they are supervising, and competent dedicated workers, pollution can be abated.

SECTION 3

CHARACTERISTICS OF THE MOSCOW RIVER WATER QUALITY ACCORDING TO HYDROBIOLOGICAL INDICES

V.A. Abakumov and G.L. Margolina

The Moscow River water quality is characterized by a combination of hydrobiological indices including indices of microbiological activity[1], primary production, saprobility, biotic potential, species diversity, and toxicological indices.

In order to obtain hydrobiological indices characterizing the water quality of the Moscow River, the qualitative and quantitative composition of bacterioplankton, phytoplankton, zooplankton, zoobenthos, and higher water plants were studied in a region of the Moscow River extending from Zvenigorod to Kolomna at the following five locations:

1. above Zvenigorod;
2. below Moscow;
3. at 107 km from the Moscow River mouth;
4. at 85 km from the Moscow River mouth;
5. in the Kolomna region (at 7 km from the mouth).

On the basis of the investigations carried out, the Moscow River water above Zvenigorod is estimated as pure with some indications of a slight pollution according to microbiological indices. The highest indices of group and species variety of plankton and benthos organisms with rapid development of oligosaprobic organisms and high biotic potential were noted here. In this region, such oligosaprobic species of phytoplankton as *Fragillaria virescens*, *F. capucina*, *Coelastrum microporum*, and *Gomphosphaeria lacustris* are abundant. Along with them, one should distinguish *Melosira granulata*, *Scenedesmus* and *Chlorella*. This region is characterized by the richness of the species composition of high water plants, number of individuals, and high protective covering. This region near the bank is characterized by an emergent plant community and a community of plants with floating leaves. Here there is an abundance of *Scirpus lacustris*, *Glyceria maxkma*, *Nuphar*

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[1] Pathogenic microorganisms are not considered here.

lutea, *Hydrocharis morsus-ranae*, *Polygonum amfibium*, *Myriophyllum verticillatum*, *Ceratophyllum submersum*, *Potamogeton lucens*, and *Potamogeton perfoliatus*. These qualitative characteristics are supported by the following quantitative indices: Number of plankton alga species - 33; number of higher water plant species - 22; number of groups of benthic organisms - 7; number of phytoplankton organisms - 369 thousand cells/liter; number of zooplankton organisms - 7 thousand specimen/m^3; number of *Oligochaeta* - 2280 specimen/m^2; relative number of *Oligochaeta* (per cent of the total number of zoobenthos organisms) - 21%; mean protective covering of higher water plants - 90%; biotic potential - 6 points; total number of bacteria - 3 million/ml; number of saprophitic bacteria - 3400/ml; number of spore-forming bacteria in 1 ml - 10; ratio of the total number of bacteria to the number of saprophitic bacteria - 900; and mg/liter of O_2 demand for a 24-hr period - 0.32. The toxicology investigations completed showed results which did not differ from the control.

Among the investigated sites, the greatest change in water quality is observed directly below Moscow. In this region of the river, the negative effect of water quality on all components of the ecological system is observed; this effect is displayed in the decrease of species variety, in the predominance of α-mesosaprobic organisms, low biotic potential, low P/R ratios, and in the increase in the number of saprophitic bacteria. Higher water plants are numerous here and *Potamogeton pectinatus* is the predominant species among them. Zoobenthos is composed of *Oligochaeta*, chironomids, and leeches, and chironomids are represented by one species only. Among plankton algae, *Melosira granulata*, *Scenedesmus quadricauda*, etc. were encountered. Toxicological investigations allow us to ascertain slight water toxicity. The following quantitative indices may illustrate the above mentioned qualitative features: Number of plankton alga species - 10; number of species of higher water plants - 4; number of groups of zoobenthos organisms - 3; number of phytoplankton organisms - 178 thousand cells/liter; number of zooplankton organisms - 31 thousand specimen/m^3; number of *Oligochaeta* - 56,000 specimen/m^2; relative number of *Oligochaeta* - 93%; mean protective covering of higher water plants - 20%; biotic potential - 2 points; total quantity of bacteria - 3.7 million/ml; number of saprophitic bacteria - 30 thousand/ml; number of spore-forming bacteria in 1 ml - 200; ratio of the total number of bacteria to the number of saprophitic bacteria - 120; and mg/liter of O_2 demand for a 24-hr period - 0.96.

The pollution level produced by such a large city as Moscow proved to be so small due to the high effectiveness of treatment installations, so that even a small river, such as the Moscow River, copes with pollution rapidly. The rapid increase in water quality at sites mentioned below verifies this fact. At the site 107 km from the mouth of the Moscow River, significant indications of an increase in water quality in comparison with a previous site are observed. This is also verified by the appearance of β-mesosaprobic indicator organisms, an increase in both the number of organisms and their species variety. *Coelastrum microporum* and *Closteium lunula* appeared among the phytoplankton. This site is characterized by the following quantitative indices: Primary production - 0.52 mg O_2/liter for a 24-hr period; demand - 1.71 mg O_2/liter for a 24-hr period; P/R ratio -

0.3; number of plankton alga species - 13; number of higher water plant species - 7; number of groups of zoobenthos organisms - 3; number of phytoplankton organisms - 2,170 thousand cells/liter; number of zooplankton organisms - 35 thousand specimen/m^3; number of Oligochaeta - 33,780 specimen/m^2; mean protective covering of higher water plants - 50%; biotic potential - 2 points; total quantity of bacteria - 3.6 million/ml; number of spore-forming bacteria in 1 ml - 200.

In the region 85 km from the mouth of the Moscow River, the water quality has considerably increased due to the development of self-purification processes. Production processes are increased with the relative decrease in destruction processes. The number of plankton organisms increases. Species variety is increased. The greatest number of species of algae and higher water plants in the lower Moscow River fow are located here. Benthic organisms are represented by five systematic animal groups, and the number of chironomid species increased to four. The indication according to saprobility of plankton forms demonstrated that they are completely devoid of polysaprobic species and that the α-mesosaprobic species approaches the minimum. Bacterial pollution is also considerably decreased. Thus, the water of the Moscow River in the 85 km region may be characterized on the whole as slightly polluted. The following data do not contradict this conclusion: Primary gross production - 2.56 mg O_2/liter for a 24-hr period; demand - 1.41 mg O_2/liter for a 24-hr period; P/R ratio - 1.8; number of plankton alga species - 20; number of higher macrophytes - 12; number of phytoplankton organisms - 9,300 thousand cells/liter; number of zooplankton organisms - 20,000 thousand specimen/m^3; number of Oligochaeta - 16,130 specimen/m^2; relative number of Oligochaeta - 80%; mean protective covering of higher macrophytes - 70%; biotic potential - 4 points; total number of bacteria - 2.5 million mg/liter; number of saprophitic bacteria - 10 thousand/ml; the ratio of the total number of bacteria to the number of saprophitic bacteria - 250.

In the Kolomna region, due to the fact that self-purification processes are developed here to the greatest extent, a further increase in water quality is taking place and indices of oligosaprobility appear. The number of organisms increased: The number of phytoplankton organisms approaches 13 million cells/liter, and zooplankton - 206 thousand specimen/m^3. The species variety of zooplankton is sharply increased mainly due to Rotifera. Production processes reach their highest intensity - 6.12 mg O_2/liter for a 24-hr period. According to all the indices of benthic organisms development, the river is also characterized by a very slight pollution; the number of zoobenthos groups - 6; the number of Oligochaeta - 1920 specimen/m^2 (lower than in the vicinity of Zvenigorod); relative number of Oligochaeta - 11%, etc.

However, the water quality in the vicinity of Kolomna does not reach the level which is observed in the vicinity of Zvenigorod. Actually, according to a number of indices, higher water quality near Kolomna is not expressed so clearly: The decrease in bacterial pollution is not so rapid; destruction processes are increased; in comparison with the 85 km outlet, the decrease in species variety for plant organisms (phytoplankton and macrophytes) is observed. Among plankton algae, for example, one species of diatoms - Melosira granulata - dominates. It comprises 80% of the total amount of algae. Macrophytes are represented by a community of water-marsh plants, which verifies the fact that the river mouth is silting in. Thus, the water in the mouth of the Moscow River in the vicinity of Kolomna according to hydrobiological indices is characterized mainly as slightly polluted.

In conclusion, it should be noted that the research carried out proves the necessity to apply a combination of water quality control methods according to hydrobiological indices which provide information not only on water pollution, but also on the state of aquatic organisms in water bodies which are valuable from the point of view of fisheries.

SECTION 4

ENDPOINTS IN BIOASSAY

Lloyd L. Smith, Jr.

INTRODUCTION

Bioassay with fish or invertebrates has long been used to determine the suitability of water for aquatic life and the toxicity or deleterious effects of industrial and domestic sewage effluents on aquatic habitat. Various techniques, exposure times, and definitive endpoints of tests to describe effects have been employed. An endpoint in a bioassay is defined for our discussion as a physiological or behavioral response to a specific concentration of a toxicant after a definite period of exposure. In order that data provided on a worldwide basis can be compared and similar conclusions derived from similar values, it is essential that a clear understanding of the usefulness of various endpoints or indicators of adverse effects be developed.

The purpose of defining specific endpoints for bioassays is to secure values in terms of milligrams per liter or degrees C which can be translated into an adequate assessment of the effects of a toxicant or effluent on fish or other aquatic species. In order that the value designated for such an endpoint have broad usefulness, it must first have a predictive capability for either acute or long-term effects of a toxicant or effluent on fish and invertebrate populations; second, it must permit comparisons of effects between different species and between different toxicants or other deleterious materials; third, it must be a practical endpoint to observe without unnecessary laboratory sophistication or excessively time-consuming analytical procedures; and fourth, it must be reported in a form that will permit comparison between laboratory and field data. Finally, an endpoint must be selected which is applicable to the particular problem. Unfortunately, much bioassay information in the world literature has not been based on careful attention to the points enumerated above. The purpose of this discussion is to elaborate on means of defining meaningful endpoints and interpreting the resultant findings.

TYPES OF ENDPOINTS

Four broad types of endpoints have been employed by various investigators at various times. They are: (1) endpoints indicating acute toxicity and resulting in death of the test organism in short-term; (2) endpoints defined by reduced fecundity, growth, or changed behavior in long-term tests at subacute concentrations; (3) endpoints defined by chemical changes in the body or changed physiological rates; and (4) endpoints defined by behavioral responses.

Acute Tests

Acute tests resulting in death are performed for short periods of time ranging from several hours to 30 days. Interpretation of results has been accomplished by calculation of a tolerance limit or concentration of the test solutions which will cause death to some proportion of the test organisms within a specified time. The median tolerance limit (LC50, TLm) or concentration killing 50% of test organisms has been employed most frequently. Two broad uses have been made of acute tests. One use has been to identify potentially toxic materials or deleterious effluents through a "screen test." This test is usually a static test of short duration, normally not exceeding 24 or 48 hr, which provides a gross estimate of probable toxicity. A second form of acute test is designated to determine a short-term response which may be used as a base to estimate effects of long-term exposures or to provide a criterion for monitoring and enforcement of water quality standards. This type of test has conventionally been of 96-hr duration, especially in the United States. More recently, longer time periods have been employed to secure a better base for predictions that will accommodate the differing speeds with which toxicants may act.

The screen test is of short-duration and must permit calculation of a median tolerance limit after 24-48 hr. The 96-hr test similarly depends on calculation of median tolerance limits of concentration over this time period, which presumably gives both a standard value and is long enough to permit acute lethal effects to develop. Because many toxicants or effluents do not effectively demonstrate their toxicity within the 96-hr period, the asymptotic or time-independent test for acute response is being used with increasing frequency. This test may run for up to 30 days and is interpreted by the flattening of the toxicity curve. It is stopped when no death occurs in test chambers for a period of 24-72 hr. This could be described as a lethal threshold concentration (LTC) (Figure 1).

Long-Term Test

A second set of endpoints can be employed in tests with long-term exposure of organisms to toxicants or effluents. The test concentrations will be lower than the median tolerance concentrations (LC50), and in consequence, the significant endpoints will usually be demonstrated by physiological inhibitions such as reductions of fecundity, growth rate, or ability to do work. Increased deaths in treatments over losses in the control after long exposure may also be used as an endpoint. Interpretation of these long-term inhibitions at low concentrations cannot be arbitrarily assessed. Usually the toxicant level which does not result in reduced growth rate, lessened fecundity, or lowered fertility is considered to be a "safe level" and consequently an acceptable concentration of potentially toxic materials. A satisfactory endpoint of a long-term test,

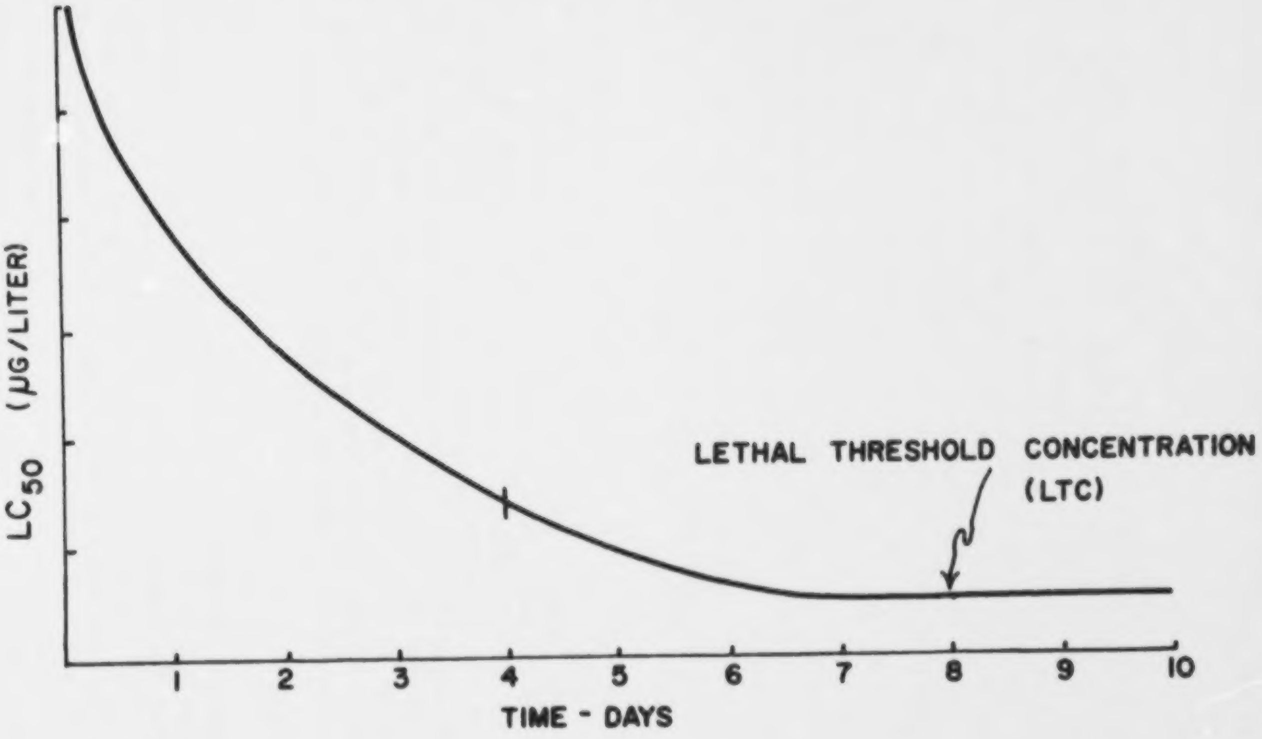

Figure 1. Toxicity curve demonstrating lethal threshold concentration (LTC).

however, may not be developed until the successful reproduction and growth rate of second-generation individuals has been shown to be unaffected.

Biochemical and Physiological Endpoints

A third group of endpoints has been explored by various investigators and has shown promise. These endpoints are related to the changes in blood characteristics and enzyme activity, changes in metabolic rates, or other physiological changes. A deficiency of this type of endpoint in long-term tests is that it usually requires sacrificing of the organisms or submitting the organism to unnatural conditions which may change metabolic responses caused by restraint of movement, excitement, or lethargy. Further, the ecological significance of these endpoints and the actual detrimental effects on the organism are difficult to interpret. Change in these parameters does not, a priori, indicate a disadvantage to survival characteristics or growth characteristics of the organisms. These endpoints have the further disadvantage that they must be described and interpreted by scientists. Tests of this kind cannot be delegated to field investigators or relatively unskilled laboratory personnel who must be employed in large-scale monitoring or surveillance programs. They have substantial value for sophisticated research and identification and classification of potential or real toxicants where results of these physiological criteria can be equilibrated with results easily interpreted in terms of ecological consequences that have practical value.

Behavioral Endpoints

A fourth type of endpoint may be described as a behavioral variation which is considered to be inhibitory to growth and reproduction or long-term survival. In various experiments it has been demonstrated that behavioral aberration will prevent spawning at toxicant levels where other measurable parameters appear to be unaffected. Changes in degree of mobility, increase or decrease of opercular ventilation, and avoidance reactions have all been used to indicate adverse physical conditions for the individual organism. As in the biochemical endpoints, interpretation of the observed results in terms of ecological and field significance is difficult unless comparative tests with other ecologically significant endpoints are made.

FACTORS AFFECTING VALIDITY OF ENDPOINT

After a significant endpoint for bioassay has been selected with careful attention to the objective sought, a series of potential limitations of the test procedure itself will determine the validity of the toxicant values produced when the endpoint is reached.

The first consideration is the health of the test species. The test fish must be in good physical health, either having been laboratory-reared under controlled and disease-free conditions or captured from a known wild stock where fish have not been stressed by pollutants or other physical factors. Disease, starvation, or careless handling before tests will seriously affect results of acute or long-term tests, regardless of which bioassay endpoint has been selected. Usually holding fish for an acclimation period in the laboratory before testing will insure a reliable response if fish remain disease free and accept food normally. It also may be desirable to subject samples of the test fish to the effects of a reference toxicant for which response has been well documented with the species.

Another factor that is important in verifying the reliability of endpoints is the degree of crowding of test organisms in the test chamber. When small fish are used, 1-2 g of fish per liter of water in the test chamber will usually allow sufficient space to permit free movement and prevent secondary effects from too many test specimens. If large fish are used, especially adults of some species, antogonism between individuals can seriously affect final results by causing fish to reach the designated endpoint at lower toxicant concentrations than fish not stressed by behavioral patterns. Test conditions which overstimulate the fish to activity or depress activity unnaturally will affect the validity of results at the selected endpoint.

Three factors influencing the validity of a selected endpoint are temperature, oxygen concentration, and pH. Temperature has a marked effect on the sensitivity of test organisms and consequently on the calculated LC50 or other endpoints selected. The effect of temperature cannot be predicted with certainty. For example, the tolerance of fathead minnows and goldfish to hydrogen sulfide is greatly increased by a 15 C lowering of temperature (Figure 2). In contrast, tolerance of bluegills is decreased by a lowering of temperature (Figure 3). It is therefore important that when an endpoint for a bioassay is selected, the test temperature is related to the objective of the test. A standard test temperature of 25 C does not necessarily relate to the ambient ecological condition in nature where the test results are expected to be applied.

Oxygen concentrations below 4 or 5 mg/liter will increase the sensitivity of test species to most toxicants. At low dissolved O_2 concentrations (below 4 mg/liter) a new stress is added that increases the adverse response of the organism to the toxicant. Similarly, extreme variations in the hydrogen ion concentration (pH) of test water can alter response and affect the validity of the endpoint chosen. This influence may be exercised through the effect of pH on ionization of the material being tested or on the physiological conditions imposed upon the fish which make absorption or blood changes more or less responsive to changes in concentration of toxicant.

A factor frequently overlooked in choosing bioassay endpoints for various fish species has been the difference in tolerance of eggs, larvae, juveniles, and adult fish. Frequently fish in the early life-history stages are much more sensitive than older fish and in consequence a satisfactory endpoint for one life-history stage will not necessarily demonstrate the sensitivity of the species through its entire life cycle. Examples of differences can be drawn from H_2S studies where frey or larvae are the most sensitive form and may vary markedly from juveniles. In con-

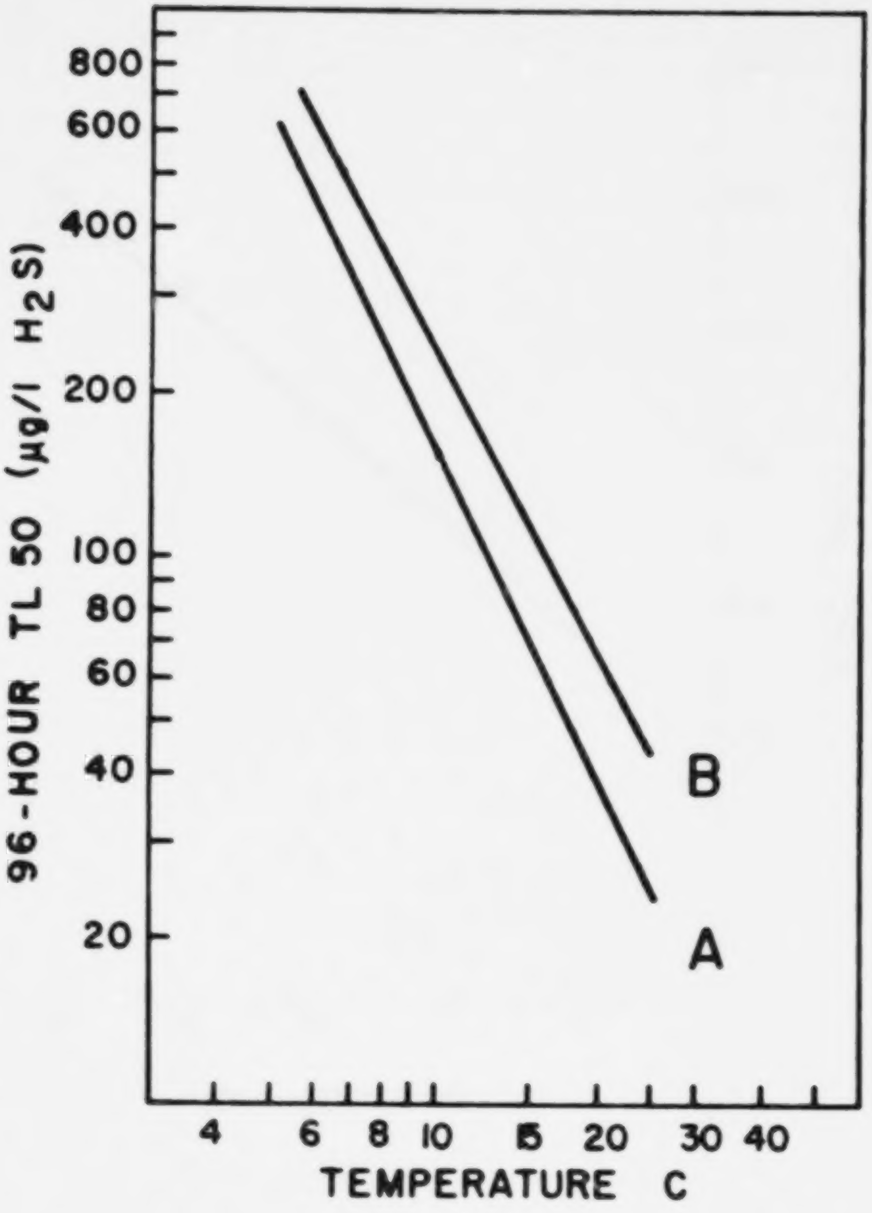

Figure 2. Tolerance of (A) fathead minnows (Pimephales promelas) and (B) goldfish (Carassius auratus) to hydrogen sulfide at different temperatures.

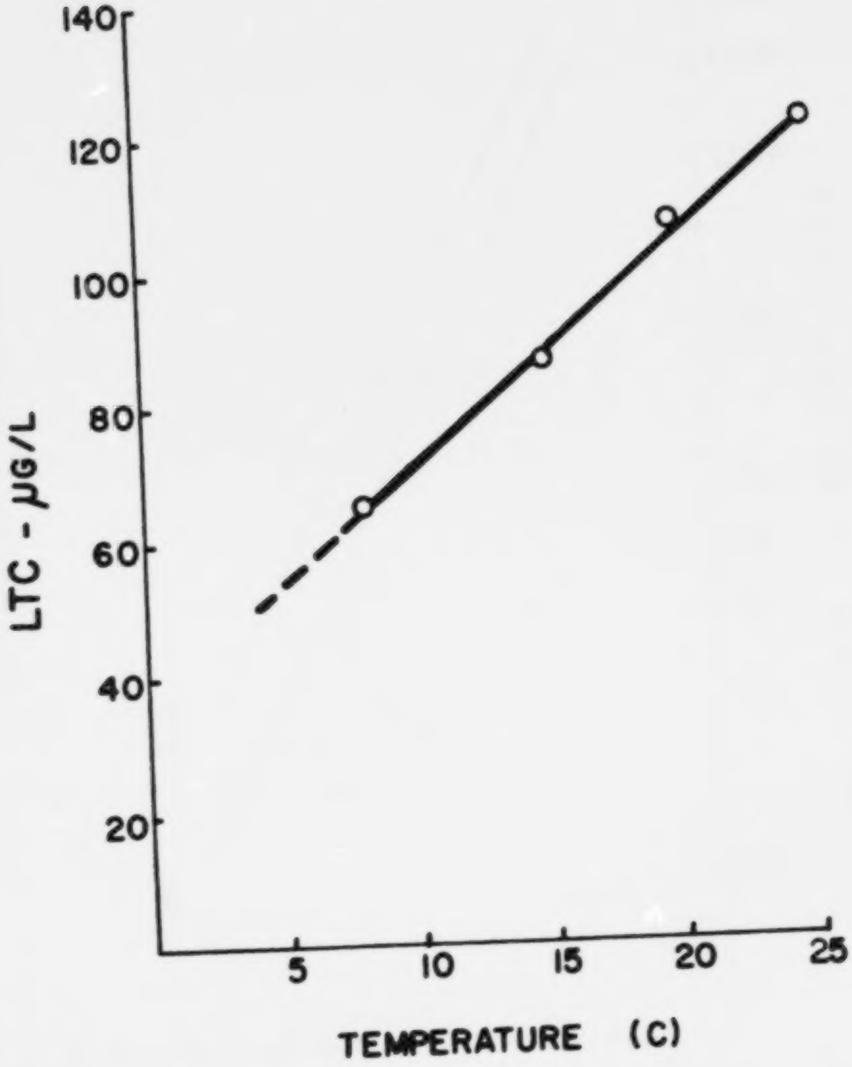

Figure 3. Tolerance of bluegills (Lepomis macrochirus) to molecular cyanide (HCN) at various temperatures. (Dotted portion of curve is extrapolated.)

trast, hydrogen cyanide (HCN) studies show that juvenile fish may be 75-80% more sensitive than the egg in some species. Further, the behavioral inhibitions on spawnig of some species like bluegill and brook trout (Salvelinus fontinalis) in respose to H_2S may be apparent at levels far below those which cause acute mortality in 96-hr or which reduce growth over long periods. These factors make it important that an appropriate life-history stage be selected for testing and that an endpoint be chosen which reflects the true sensitivity of that stage to the toxicant.

Selection of test fish stocks from widely separated geographical areas or different cultured stock may introduce wide variations in results. In fathead minnows acute sensitivity to cyanide (HCN) and H_2S may vary as much as 30-40% between stocks. It is also important that the influence of test conditions (temperature, pH, O_2, fish numbers) be taken into consideration when selecting an appropriate endpoint for bioassay and in evaluating the results when they are obtained.

PREDICTION OF LONG-TERM ADVERSE EFFECTS FROM SHORT-TERM TESTS

Ideally a toxicant should be administered to a fish or an invertebrate throughout its entire life history, beginning with the egg through reproduction and into the second generation, to determine concentrations which will not adversely affect the population. However, the large number of known toxicants and unknown mixed toxicants which must be tested will prevent definition of safe levels for many materials by long-term tests. It is therefore common practice to make an acute test (96 hr) defining some median tolerance limit (LC50 or TLm) and then to apply a mathematical factor which will reduce the value of this concentration to that considered safe for completion of all life-history stages. This factor is usually called an application factor and is calculated by dividing the safe concentration by the 96-hr LC50 (TLm) of the toxicant. Historically, first approximations of this factor were made by comparison of acute median tolerance limit tests and long-term or chronic tests of a limited number of toxicants. The tests were conducted on the same species and under the same conditions. Results of acute tests were also compared to concentration of toxicants in streams which contained normal fish populations. While many materials appear to have application factors of similar magnitude, certain families of materials have factors different by orders of magnitude from established means and factors (Table 1). With a single toxicant the application factor may vary substantially between species. An example can be drawn from our studies of H_2S in which the application factor varies widely between species and between life-history stages of the same species (Table 2). Also fish species react very differently to materials in similar chemical families (Table 3). These examples indicate that no single application factor can be used to predict "no-effect" toxicant concentrations from short-term tests. In consequence, uses of application factors with short-term tests have tended to be more restrictive in their results than necessary in some cases and much less restrictive than required in others.

TABLE 1. COMPARISON OF APPLICATION FACTORS FOR VARIOUS COMPOUNDS

Species	96-hr LC50 (μg/liter)	MATC[a] (μg/liter)	Application factor
Malathion (Organophosphorus pesticide)			
Fathead minnow	9,000	200-580	0.02-0.06
Bluegill (Mount and Stephan, 1967)	89	3.6-7.1	0.04-0.09
Guthion (Organophosphorus pesticide)			
Fathead minnow (Adelman, Smith, and Siesennop, 1976)	9,000	0.33-0.55	0.00017-0.00027
Cyanide			
Fathead minnow (Smith, unpublished)	107	5.3-17.3	0.049-0.162
Cadmium			
Fathead minnow (Pickering and Gast, 1972)	7,200	37-57	0.005-0.008
Bluegill (Eaton, 1974)	24,000	31-80	0.0015-0.0039
Copper			
Fathead minnow			
Soft water (Mount and Stephan, 1969)	84	10.6-18.4	0.13-0.22
Hard water (Mount, 1968)	430	14.5-33.0	0.03-0.08
Brook trout			
Soft water (McKim and Benoit, 1971)	100	9.5-17.4	0.10-0.17

[a] Maximum allowable toxicant concentration.

TABLE 2. APPLICATION FACTORS FOR H_2S WITH JUVENILES OF SIX FISH SPECIES (EXPRESSED AS μg/LITER)

Species	LTC	Chronic no-effect	Application factor
Brook trout	16	6	0.375
Rainbow trout	9	3	0.353
Fathead minnow	16	3	0.188
Goldfish	71	5	0.070
Walleye	19	3	0.158
Bluegill	32	1	0.030

TABLE 3. 96-HR LC50 OF EIGHT FISH SPECIES TO PESTICIDES (EXPRESSED AS μg/LITER)[a]

Species	DDT	Parathion	Guthion
Ictaluridae			
Catfish	16	5,710	3,290
Cyprinidae			
Goldfish	21	9,000	4,270
Carp	10	7,130	695
Centrarchidae			
Sunfish	5	5,170	52
Black bass (largemouth)	-	5,220	5
Salmonidae			
Rainbow trout	7	2,750	14
Coho salmon	4	5,300	17
Percidae			
Yellow perch	9	3,060	13

[a] Macek and McAllister, 1970.

SUMMARY

On the basis of the foregoing considerations, it is recommended that (1) the most sensitive stage be used where possible as the basis for acute median tolerance limit tests; (2) temperature used for tests should approximate the natural conditions to which the fish will be subjected during critical periods in the outdoor environment; (3) median tolerance limit value should be based on the time at which an asymptote is reached in the decline of the toxicity curve; (4) where possible, reproductive behavior and success be used as the final criterion of the proper endpoint used to determine safe concentration of toxicants or effluents; and (5) uniform application factors not be used over a broad spectrum of species or toxicants without definitive chronic tests.

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SECTION 5

PHYSIOLOGICAL-BIOCHEMICAL ASPECTS OF WATER TOXICOLOGY

V.I. Lukyanenko[1]

The most important biological and economic problem for an industrialized society today is the problem of "clean water". This problem stems from the increasing rate of water consumption and the rising pollution of inland water bodies by wastewaters, oil and oil products, and pesticides. Currently the volume of polluted wastewater discharged into our water bodies is approaching 700 cubic kilometers. There are thousands of substances in this wastewater which are toxic to organisms in some way or another. During the last decade, the problem of preventing pollution in inland water bodies became still more complicated due to the wide-spread use of agricultural chemicals for pest control and plant protection. The list of pesticides used in agriculture grows continuously larger. World production of these toxicants in now approaching 1.5 million tons/year.

During the twenties and thirties of this century, a general concept of protecting a water body from pollution was formulated both in our country and abroad, so as to restrain the progressing chemical pollution of water bodies and guarantees the multiple use of water resources. In accordance with this concept, state regulation of wastewater discharge and control of pollution by establishing maximum permissible concentrations (MPC) of harmful substances discharged into water bodies was introduced. For quite understandable reasons, the dominant role in solving the problem of protecting water bodies from pollution and establishing the MPC in water bodies in our country belonged to the field of health specialists.

Medical specialists have done a considerable amount of work on this problem. They have developed ideas on hygienic criteria for the harmful effects of wastewater; strengthened the physiological-biochemical direction in studies of water hygiene and sanitary protection of water bodies, and at present have experimentally proven the MPC of about 300 harmful substances introduced into water bodies that exclude unfavorable effects of these substances on people's health (Cerkinskiy, 1949; Cherkinskiy, Krasowskiy, 1967). However, after a short period of time, it was learned that the MPC of many substances (salts of heavy metals, insecticides) which fully satisfy the health specialists do not guarantee the purity of water bodies from a general biological standpoint or from the fishing industry's point of view.

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[1] TsNIORH, Astrakhan, USSR.

The MPCs for many substances were found to be significantly lower for fish and other organic organisms than for man. What is considered harmless for man proves to be fatal for fish, especially under chronic exposure. The lower tolerance of fish and other aquatic organisms to pollutants discharged into water bodies is quite understandable because the polluted water is their habitat.

All these facts drew our attention to the need for biological standardization of harmful substances in tests on fish and other aquatic organisms in order to retain the normal course of biological processes and a high biological productivity in them. But the solution to this central water toxicology problem is senseless without detailed studies of character and means of influence of harmful substances on the vital activity of aquatic organisms on the whole, and fish in particular, according to differences in their organization, and taking into account their high sensitivity to many toxicants of inorganic and especially nature. Therefore, understanding the effect of toxicants on fish and other aquatic organisms is a necessary prerequisite for developing scientific bases and methods for determining MPCs which are applicable to the problems of biological standards. We are talking about a physiological-biochemical approach to solving the basic problems of water toxicology, in research to develop biological standards for protecting water bodies used by the fishing industry.

The first stage of research was to show the similarity and reaction properties of fish as cold-blooded vertebrates to various toxicants in comparison with warm-blooded vertebrates. As our studies on the phenol intoxication fish model, at the USSR Academy of Sciences, Institute of Biology of Inland Waters in 1961-1964 and continued at the Central Sturgeon Research Institute, showed, there are various pathological changes in fish organs which affect the many physiological systems and which precede the death of poisoned fish. Examples are a disturbance in the behavior reflex activity, a disturbance in respiration, changes in the electrocardiogram, changes in the activity of tissue cholines-terase and ammonia content in the brain, decrease in hemoglobin concentration and increase in blood sugar, changes in albuminous content of blood serum, changes in ESR, and a series of other hematological changes (3-8).

In the last few years, other laboratories have begun to follow this direction, have added new data (9-18) on a variety of fish reactions and ways toxicants influence aquatic organisms. All the experimental data available today permits us to confirm that fish reactions to various groups of toxicants, by direction as well as by content, are mainly similar to those reactions known for warm-blooded vertebrates. Basic principles and methods for assessing toxicity developed by general and sanitary toxicology results could, therefore, be used for aquatic toxicology. This is why we think that the MPC of a harmful substance for fish, just as for higher terrestrial vertebrates, should not exert a toxic effect on any of the numerous facets of its vital activity.

In other words, the MPC should not exert a toxic effect on any of the numerous functions of the organism since the disturbance of any of the functions might lead to the disturbance of the physiological normal level of the organism and its biological well-being (3). Such an attitude is quite natural for a physiologist, since from the physiological point of view, any one of the numerous functional systems is equivalent and indispensable for the normal activity of the whole organism, and the persistant disturbance of each of them will inevitably destroy the activity of the others, including the reproductive function.

According to this point of view, we have formulated ideas on toxicity criteria in water toxicology. We must consider as toxic (threshold) a concentration which evokes some expressed pathological change in any of the functional systems of an individual organism, since persistant disturbance of the activity of any physiological system, whether it be blood circulation or hemopiesis, respiration or nutrition, behavior or reproduction, sooner or later leads to irreversible homeostatic disturbances and finally to the destruction of organisms.

It appears from the above that the threshold concentration value determined from fish toxicological investigations to develop criteria, depend largely on how correctly we identify the function affected, i.e., the adequacy of methods for estimating pathological changes in the activity of the functional system. Therefore, the search for more sensitive, specific methods with high resolution which permit us in a very short time to obtain scientifically substantiated MPCs for harmful substances, is of primary importance. Already MPC values for almost a thousand various substances discharged into water used for fisheries are needed.

The solution of this colossal problem in a very short time is possible only with the aid of more sensitive contemporary physiological and biochemical methods for monitoring the functional state of test fish and other aquatic organisms. These methods surpass 10-100 times the "fish trial-and-error method" due to their resolution capabilities. The selection of the specific method to determine the MPC value of any harmful substance must be based on the knowledge of toxicodynamics of the substance under investigation and the understanding of the effect of various toxin groups, i.e., a clear identification of the most sensitive target function. Here is an example to explain this thesis.

For example, a large group of substances of an organic nature (toxins of the phenol series, many pesticides, dyes, etc.) cause a complicated symptom-complex of intoxication in fish. This permits us to assume the influence of these substances is on the central nervous system. However, this required direct experimental proof and it was obtained (4, 3, 19-21) using the model of phenol intoxication in fish. In a number of experiments beginning in 1962-63 at the USSR Academy of Sciences Institute for Biology of Inland Waters, the dominant role of the central nervous system in the development of the complicated symptom-complex of intoxication in fish with toxins of the phenol series has been proven. For example, we succeeded in completely cutting off the first phase of phenol intoxication--rapid motor activity--in anesthetized crucians (novocain, urethane). In other words, generalized inhibition of the central nervous system, resulting from narcosis, prevents the development of the most typical symptoms of phenol intoxication in fish. A further pharmacological analysis of phenol effects on fish was carried out using curariform preparations (succinilcholine, phlaxedil, paramyon) possessing pronounced blocking action on neuromuscular conductivity in the myoneural synapse. The experiments indicated that phenol does not exert a direct stimulating effect on the muscles of the fish body, and that the nerve impulses arising from the central nervous system are the basis for motor reaction of fish under the influence of phenol. This is indicated by the inhibition of motor reaction in phenol-exposed fish by means of a pharmacological disturbance in the neuromuscular nerve impulse transmission within the region of the myoneural synapse. In this respect succinilcholine, from the group of preparations producing stable depolarization of the terminal motor plate, was the most effective. K. Kuba's (22) electrophysiological work completely confirmed the results of our experiments. He also concluded that the myoneural synapse was one of the points in which phenol action plays a dominant role.

We obtained new evidence indicating the dominant role of the central nervous system in the reaction of fish to phenol stimulation, and in particular, of stimulation of the brain in fishes with several spinal cords (operative disconnection) and on an isolated head preparation (4, 3). The brain proved to be prominent in the development of more characteristic components of the reaction of fish to the toxic effect of organic toxins. Motor activity occurred at the beginning and spasms later.

After the complete removal of the brain, not one of these reactions developed. The spinal cord is the most important link in the reflex arch, the conductor of the impulses which are caused by phenol stimulation, from various branches of the brain to peripheral neuromuscular systems in fish (3).

Naturally, the question of the specific phenol effect on the central nervous system in fish was been raised. A partial answer to this question was found during experiments with anticholinesterase preparations (phosphacol, neostigmine, physostigmine). The preliminary injection of these preparations into test crucians fully inhibited the external symptom-complex of phenol intoxication in fish. Experiments with anticholinesterase preparations led to the conclusion that the dynamics of an acetylcholine metabolism and, first of all, of a system of acetylcholine-cholinesterase in cholinergic synapses of the central nervous system and neuromuscular synapses, plays a dominant role in the development of the complex of reactions caused by phenol (4). Confirmation of this point of view came from biochemical data on changes in the muscular cholinesterase activity under the influence of phenol (5), as well as from electrophysiological data. According to the latter, phenol increases the amplitude of the stimulating synaptic potential and causes the appearance of tiny potentials on the terminal plate, i.e., it facilitates neuromuscular transmission. It is fascinating to explain the biphase course of phenol intoxication in fish, namely, the intitial highest motor stimulation with subsequent spasms and paralysis, in light of the dynamics of change in the acetylcholine concentration in cholinergic structures of the central nervous system and in myoneural synapses. The initial highest central stimulation caused by phenol appears to be connected with the stabilization of "physiological" acetylcholine and its accumulation in cholinergic synapses. Central paralysis, coming after the stimulation and caused by phenol, might be understood as the result of the accumulation of acetylcholine in brain synapses in ordinate pessimal concentrations which cause the inhibition. Certainly, the acetylcholine accumulation in synapses might be caused by two methods--either due to cholinesterase inactivation or due to an increase in acetylcholine quanta isolation from nerve endings, but most likely both processes take place. However, it is not of particular concern, since both methods lead to acetylcholine accumulation in synapses in pessimal concentrations.

Such are the basic results of the experimental studies into the mechanisms of phenol effect on fish, which we attempted in the early sixties. They permitted us (4) to substantiate prospective use of the behavior-reflex method during ichthyotoxicological experiments as the most sensitive tests for determining the chronic effect of trace concentrations of various homologues of the phenol series and for determining the MPC value of this group of substances. Experimental data obtained by B.A. Flyorov and V.E. Matey in their experiments on gold crucians and Lebistes groups (23-26) fully supported this point of view. As should be expected, pathological changes in behavior reflex activity in fish occur long before the appearance of expressed phenol intoxication symptoms and are observed in concentrations that are 5-8 times lower than acute toxic concentrations of this substance. There are good reasons to believe that the behavior reflex method will hold a fitting place among other methods for determining the MPC of various groups of organic toxins with expressed activity on the central nervous system which is characteristic for them.

The growth of international cooperation in the field of water toxicology and ichthyotoxicology brings our attention to the question of unifying methods for estimating the degree of toxicity for various groups of harmful substances, and standardizing experimental conditions and principles for interpreting test data. At present, this is quite possible, thanks to the accumulation of data concerning the dependence of the results from ichthyotoxicological experiments on many variables. In this case, two groups of factors characterizing both fish habitat (chemical water state, oxygen content, pH value, water temperature, etc.) and the test object itself (species, age, and sexual properties of fish sensitivity and stability as well as initial functional state) play a dominant role. Therefore, in order to develop an actual and potential toxicity for a harmful substance, it is necessary to carry out experiments which allow for fluctuations in physical-chemical parameters of the water medium (27), i.e., experiments carried out at a relatively high temperature and a moderately low oxygen content. Water hardness and pH value are selected in such a way as to develop the highest possible toxicity for the substance under investigation. The most sensitive species of fish of the ichthyofauna under investigation (28) should be used as the test object. In this case, it is important to consider the sensitivity of the test species at various stages of ontogenesis, and choose the most sensitive one (29, 3). Together with the physical-chemical factors of the water environment and the biological properties of the test object, the exposure time plays a dominant role in determining an ichthyotoxicological experiment. Depending on the specific problem before an investigator and on the degree to which the substance of interest is studied, the duration of an experiment must be limited to 24-96 hours (acute experiment), to 10-30 days (subacute experiment), or 1-3 months (chronic experiment). The duration of an experiment is determined by the resolving powers of the research method used by an investigator. The higher the sensitivity and resolving powers of a method, the shorter the time for determining the toxicity of a substance and the MPC value. Just as the physiological-biochemical methods for toxicity determinations are different, the mechanisms of the effect of various toxin groups are not the same (30). There is no doubt that unifying methods for estimating the degree of toxicity of substances and determining the MPC for these substances will bear fruits in the near future and facilitate the solution of the basic problem of water toxicology which faces all water toxicologist--of limitation biologically harmful substances discharged into water bodies--by determining the MPC. The solution of this problem is possible only on the basis of a synthesis of the ideas and general and sanitary toxicology methods with the achievements of modern physiology and biochemistry of fish and other aquatic organisms (31).

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SECTION 6

BIOENERGETIC AND OTHER CONSIDERATIONS IMPORTANT IN THE STUDY OF WATER QUALITY INFLUENCES ON FISH GROWTH

Peter Doudoroff

The determination of maximum permissible concentrations of water pollutants in waters in which fish must be adequately protected has long been a major objective of physiological, toxicological, and ecological research in the field of water pollution in the United States and in the Soviet Union. We have fully recognized that prevention of fish mortalities is not alone a sufficient goal of pollution control directed toward the protection of fisheries, for unimpaired fish production clearly depends upon adequate reproduction and normal growth of fish, and not only on their survival. However, research into the effects of water pollutants on fish growth has been limited, and much of it is rather crude or superficial and not very helpful in the prediction of effects under natural environmental conditions. Effects on reproduction, which certainly is essential to fish production, have received more attention. But some interference with reproduction of fish may sometimes have relatively little or no effect on their production, because numbers of young produced remain sufficient for nearly full utilization of the available habitat and food resources. Production (elaboration of new tissues) may even increase under some circumstances, because reduced competition for food permits a larger portion of the food materials and energy to be utilized for accelerated growth and less for maintenance metabolism. On the other hand, any interference by pollution with the growth of the young must necessarily result in impairment of production.

To understand effects of environmental factors on the growth of animals, it is essential to consider to what extent and to what ends the energy and materials of food consumed by the animals are utilized and how they are distributed, under the different conditions, among portions having different fates. It is not my purpose to expound in depth here on the principles of the science of bioenergetics and their application in the study of the growth of fish, a field of research to which Soviet scientists, notably the late V.S. Ivlev and also G.G. Vinberg, have made outstanding contributions. Nor can I treat fully the influence of water pollutants on fish growth. A more detailed exposition of the principles involved and their applications in pollution research can be found in the chapter on "Bioenergetics and Growth" of Warren's (1971) "Biology and Water Pollution Control" (p. 135-167). My purpose here is, mainly, to propose and explain a general scheme of procedure, based on important bioenergetic and ecological considerations, for efficient experimental investigation of undesirable effects on fish growth of water pollutants, especially toxic ones. The proposed studies are directed toward reasonably reliable estimation of limits of water quality alterations having virtually no such harmful effects in nature. Because of the unavoidable complexity and difficulty of sufficiently thorough investigations directed toward the stated goal, it is clearly desirable to minimize the number of the most difficult and costly experiments to be performed. In the plan of investigation suggested here, the sequence of different experiments will provide, in my opinion, for maximal efficiency of the studies of a practical nature, which should not be expected to lead to complete understanding of observed effects of pollutants.

A reduction of growth rates of fish can be a consequence of any one or more of the following effects of degradation of water quality: (1) reduction of the available food supply; (2) impairment of the appetite of the fish for food; (3) reduction of the feeding activity of the fish and their ability to find and capture their prey; and (4) impairment of the efficiency of metabolic utilization of food by the fish and its conversion into body tissues. We must look for each of these possible effects.

Maximum concentrations of toxic water pollutants having no material effect on over-all food resources are very difficult to determine experimentally, because most fish depend, directly or indirectly, on a large variety of aquatic organisms for their food supply. Demonstrable adverse effects on the reproduction and growth of some food organisms may not be assumed to bring about a lower availability of food, since the production of other, more tolerant species that can be utilized by fish may increase as competition for space and food by the less tolerant species declines. In waters polluted with energy-rich organic materials, such as pulp mill wastes, the abundance of fish foods in the aggregate sometimes even increases, while some disappear, because the growth of some microorganisms that serve as a primary food resource for invertebrates is stimulated. Often, therefore, reliable prediction of effects of pollutants on the availability of fish foods in natural habitats can be achieved only through experiments performed under nearly natural conditions, such as experiments with artificial or modified natural streams in which complex plant and animal communities can be maintained. The results of such costly experiments often are difficult to interpret if there are no good reasons to believe that any observed impairment of the growth of fish could have been due only to a reduction of availability of foods and not to one of the other possible causes mentioned above. Obviously, it is not with difficult experiments of this kind that one should begin in seeking to reduce the number or range of concentrations of a pollutant that need to be tested in other additional experiments.

On the other hand, the maximum concentration of a toxic substance having no pronounced adverse effect on the appetite of juvenile fish, or on the highest rate of food consumption of which they are capable, or on their efficiency of utilization of food resources if their activity is not materially depressed by the poison at that level, can be quite easily determined through laboratory experiments. To find the level that does not impair the appetite, it is necessary only to expose groups of fish for sufficiently long periods to different concentrations in small aquaria, to supply them continuously or frequently with as much attractive, palatable, and nutritious food (preferably live food) as they will consume, and to measure the food consumed. The fish, held preferably in individual aquaria or compartments, should be uniform in initial size and carefully weighed. By weighing accurately the food offered and the uneaten food removed from the aquaria at daily or other suitable intervals, the mean daily consumption (in grams per gram of fish) at each tested concentration of toxicant can be determined and compared with that of controls. The gross efficiency of food conversion can be determined by dividing the gain in weight of the fish during an experiment or a suitable time interval between weighings by the weight of food consumed during that period. This efficiency can be expected to be reduced, as compared with that of controls, when the food-consumption rate is markedly reduced. If it is found to be reduced also at a concentration of a poison at which the food consumption is not reduced, impairment of metabolic processes is indicated, and lower concentrations must be tested to determine the highest one at which no such effect is demonstrable. The duration of the food-consumption and growth tests need not exceed a month and can be much shorter when growth is rapid, but it is advisable to expose fish for a fairly long time before final measurements of their growth and food intake are made, especially when substances known to be accumulative poisons are tested.

Any concentration of a poison at which the food intake is found definitely to be reduced can be taken to be level at which the growth of the fish probably would be impaired under natural conditions whenever the availability of food is not a limiting factor. This statement, or proposal, can be reasonably countered, however, with the objection that the food consumption and growth of fish in their natural environment generally are limited by the availability of food and not be the appetite of the fish. One may well argue that, for this reason, the concentration of a poison at which food consumption begins to decline in aquaria where food is so abundant that the fish can obtain as much as they can eat with little or no effort is essentially meaningless. In addition, it is doubtless true that the annual food consumption of fish in nature is, as a general rule, if not always, far less than their annual assimilative capacity. At natural temperatures growth rates of well-fed fish in laboratory can greatly exceed those usually found in nature, where losses of weight during periods of food shortage are not unusual. Perhaps few biologists who have studied the growth of fish believe that the availability of food ever is not a limiting factor for any considerable periods of time. But it seems to me not unreasonable to assume that in some very productive natural environments the rates of food consumption are not limited by food availability during some fairly extended periods of maximal or nearly maximal abundance of food in the season or seasons in which most of the growth of fish takes place. An inability of fish to take full advantage of a temporary abundance of food in such a situation could have a considerable effect on their annual weight gains.

Whenever there is sufficient reason to reject the proposition that the growth of fish in a given environment is not food-limited at certain times and for periods long enough to be of consequence, as when that growth is known to be always much slower than that of controls in the proposed laboratory tests, a different alternative approach can be used. Each fish in the aquaria then can receive daily a uniformly restricted food supply that is believed or assumed to be not much less than the maximum amount of food consumed per day by individuals of the same size in the natural environment, excepting rare occasions. The maximum concentration of a poison at which this restricted food ration is fully consumed by the experimental fish and their growth is not demonstrably impaired, as compared with that of controls, than can be determined. In all such experiments the food should be as much as possible like natural foods; for comparative purposes, amounts of these foods should be expressed not in grams but in caloric equivalents. Water temperatures normal for the season of maximum food consumption in nature should be maintained during these tests. Various methods for the estimation of rates of food consumption by fish in their natural environments have been described (Davis and Warren, 1968). Estimates of amounts of food consumed during short intervals of time probably cannot be very reliable. Average rates of growth of fish in nature during longer periods (seasons of the year) can be evaluated through appropriate observations, and their average daily food consumption during these periods can be estimated through laboratory experiments by which rates of growth in aquaria are related to food-consumption rates. In the absence of contrary information, the assumption then can be made that a food-consumption rate twice the average seasonal rate is a rate that should be attainable but does not have to be exceeded at any time during corresponding season if growth in the natural environment is not to be materially impaired. Accordingly, a daily feeding rate twice the estimated, average, natural food-consumption rate during the season of maximum food intake can be taken to be appropriate for the proposed toxicity tests. Admittedly, this recommendation is somewhat arbitrary, for frequent fluctuations of natural food availability and consumption may be sometimes much smaller and sometimes greater than those that it implies, but I believe that it cannot lead to serious error in the estimation of maximum harmless levels of water pollutants.

When an unrestricted food supply is provided to the experimental and control fish, as first suggested, it will sometimes be found that fish exposed to toxic substances consume more food, and not less, than the controls. In this way they may compensate partly or wholly for a reduction of the efficiency with which the consumed food can be utilized, so that growth in the aquaria may be reduced little or not at all. But such compensation is possible only when food is extremely abundant and available. It is certainly true that the growth of most fish in nature is limited, at least during a large part of each year, by the limited availability of food, which renders the compensation impossible. In their natural habitat poisoned fish are likely to find and capture less of their prey than normal fish would when there is a shortage of food. Therefore, any reduction of the efficiency of food conversion in fish affected by poisons must result in impairment of their growth whenever the density of their prey is low enough to be a limiting factor.

Increases of food consumption by fish together with impairment of the efficiency of their utilization of food in aquaria in which the food was abundant have been observed in experiments on the effects of sublethal concentrations of sodium cyanide (Leduc, 1966) and of potassium pentachlorphenate (Chapman, 1965; Warren, 1971, p. 163-164). Cyanide levels not far below lethal levels depressed the food conversion efficiency (gross) of cichlids, Cichlasoma bimaculatum, by as much as 35% on the average, but caused increases of the amount of food consumed during the 36-day tests that averaged as much as 30%. Potassium pentachlorphenol (0.2 mg/liter) at first depressed the food-consumption rate of these fish, but later in the 42-day tests the consumption rate of the poisoned fish was much greater than that of controls. Growth rates were initially depressed by both the cyanide at high concentrations and the pentachlorphenate, but before the tests were concluded the fish exposed to the poisons were growing even faster than the controls. Averaged weight gains of the experimental and control fish during the entire experiments therefore differed little or not at all. When food rations were uniformly restricted, however, the growth of fish in the pentachlorphenate solutions was decidedly less than that of controls because of the deranged metabolism and reduced efficiency of food conversion.

Whenever the food consumption of fish that are given all the food that they can eat is found to increase in the presence of the toxicant studied, the food supply should be uniformly restricted so that all of the fish, including the controls, will consume all of the food offered. The restricted daily ration in one series of tests should not be much less, however, then the maximum ration found to be consumed by all the fish. The highest concentration of the toxicant that does not demonstrably impair the growth of the fish receiving this fixed ration should be determined. The impairment of food-conversion efficiency by poisons can be much more pronounced when the levels of food availability and consumption are high than when they are low. Sometimes, however, the effect in question may be more pronounced and readily demonstrable at low levels of food availability and intake than at high levels. Therefore, in looking for possible interference with food-conversion efficiency, it is always advisable to perform an experiment in which a small, uniformly restricted ration of food is provided to each fish, regardless of whether the appetite of the fish has or has not been found to be stimulated by the poison tested. This ration should not be much greater than the maintenance ration for the controls, which is the ration that brings about neither growth nor loss in weight of these fish. Should the food-conversion efficiency of fish receiving the small, restricted ration be found to be impaired more markedly by a poison than that of fish consuming much larger amounts of food, tests with this small ration should be performed to determine the highest concentration of poison that does not impair the conversion efficiency. Ideally, such series of tests of different concentrations are performed at four or more levels of food availability, ranging from unrestricted supplies to restricted supplies barely sufficient or insufficient for maintenance of the initial body weight of controls (Warren, 1971). Such more laborious experiments certainly can be very instructive and can increase confidence in the reality of observed small differences of food-conversion efficiency. They have not been shown, however, to be absolutely necessary for achieving the not very ambitious objective of the practical investigations proposed here.

The maximum concentration of a toxic pollutant that impairs neither the appetite nor the food-conversion efficiency of the experimental fish so much that their growth under natural conditions must be judged likely to be seriously affected having been determined, what should be the next step? May we now assume that this concentration can affect the growth of the fish adversely only if it causes a reduction of the natural food supply? This assumption is not usually justifiable, because an impairment of water quality can reduce also the activity of animals and, consequently, their success in seeking, pursuing, and capturing their prey, as well as in eluding their enemies. Experiments have shown that exposures of salmonid fishes to exceedingly low concentrations of sodium cyanide, for example, has a very pronounced, rapidly produced, and lasting effect on their swimming ability. Though not known to be otherwise demonstrably affected, they become unable to resist currents of moderately high velocity nearly as long as individuals not exposed to the cyanide (controls). Such effects may or may not interfere with normal feeding activities. In experiments with other fish that proved less susceptible, *Cichlasoma bimaculatum*, Leduc (1966) found that the duration only of moderately rapid swimming, and not that of very rapid swimming, was reduced by exposure of the fish to low cyanide levels. A reduction of maximum swimming speeds sustainable for long periods of time cannot be said obviously to impair the foraging efficiency of fish; only short bursts of speed in the pursuit of prey are commonly observed and are clearly essential to successful feeding of many species. Still, any interference by toxic substances with the ability of fish to exert themselves may, in some subtle way, cause food consumption under natural conditions to decline, thus reducing growth. Some poisons may interfere mainly with very rapid swimming.

In experiments with artificial, concrete-lined ponds stocked with known numbers of mosquitofish, *Gambusia affinis*, and several largemouth bass, *Micropterus salmoides*, which fed on the mosquitofish, the food consumption and growth rates of the bass were decidedly reduced when dissolved oxygen concentrations were reduced (Brake, 1972; Warren, Doudoroff, and Shumway, 1973). The bass consumed fewer of the mosquitofish and grew less in a pond with a moderately reduced oxygen concentration than in a control pond, even though laboratory tests had shown that they were capable of consuming much more food and of growing much faster at the same oxygen concentrations and temperatures when the food was more available. The mosquitofish were provided with artificial cover (rolls of wire netting placed in the shallow water near the periphery of each pond) and were not easily caught by the bass. Consequently, the food-consumption and growth rates of the bass even at high oxygen concentrations were dependent on the density of the prey, that is, they increased when the number of mosquitofish placed in the ponds was increased. Aquarium tests had shown that the appetite of the bass, or the amount of food that they are able to consume when the supply of food is unlimited and the prey easily captured (not protected) is reduced at moderately low oxygen concentrations just as the food consumption in the ponds was reduced (Lee, 1969; Warren et al., 1973). The impairment of appetite could have been somehow partly responsible for the reduction of food consumption at the low oxygen concentrations in the ponds. However, since the bass in the ponds were obviously unable fully to satisfy their appetite for food at any of the tried oxygen levels, this reduction of their food consumption must have been due primarily to their reluctance to expend as much energy in pursuing their prey at the low oxygen levels as they expended in the presence of more dissolved oxygen.

The amount of energy that the bass expended in capturing and assimilating their food in the ponds at like temperatures and high oxygen concentrations was virtually independent of the density of the prey. In other words, the average rate of their metabolism was not appreciably affected by changes in availability of the food. As food became less available, so that less was consumed, less energy had to be expended in the process of assimilation of the food ingested and more could be expended, therefore, in pursuing the prey, but the total energy expenditure did not increase or decrease demonstrably. The average metabolic rate of the bass in the ponds was estimated by the energy balance (or caloric apportionment) method. The caloric value of the unassimilated portion of the food and of metabolic wastes (estimated through laboratory experiments, using small aquaria) and the measured increment in caloric value of the bodies of the growing bass were subtracted from the caloric value of the food found to have been consumed by the bass during an experiment; the remainder, in calories, was then divided by the mean caloric value of the bodies of the bass, in kilocalories, and by the duration of the experiment, in days. Fairly uniform values of about 26 cal/kcal per day were thus obtained at temperatures near 21 C (Lee, 1969; Doudoroff and Shumway, 1970).

At moderately reduced oxygen concentrations the feeding activity of the bass was reduced evidently because the metabolic rate was limited by the oxygen supply, and this must have been the reason also for the reduction of the appetite of the bass in aquaria at the same oxygen concentrations. When held in aquaria with an unlimited food supply (easily captured mosquitofish), the bass had at high oxygen concentrations an average metabolic rate approximately equal to that of more active bass in the ponds. Reduction of the oxygen concentration thus can be expected to limit the appetite of fish whenever it impairs their feeding, and vice versa. Effects of some toxic substances on feeding activity and appetite may be similarly related, but those of other poisons may be quite unrelated phenomena. It is highly probable that some toxic substances, at concentrations that impair neither the appetite nor the gross food-conversion efficiency of fish held in aquaria or even improve one or both of them, nevertheless reduce the rate of growth of the fish under natural conditions by limiting their feeding activity. Such an effect has not yet been clearly demonstrated, probably only because the appropriate experiments have not been performed. But we surely may not assume that a reduction of feeding activity will always be accompanied by an impairment of appetite as it apparently is when oxygen concentrations are reduced.

As I have pointed out already, cyanide poisoning can greatly restrict one kind of activity of fish, at least, while causing an increase of their appetite for food; for reasons to be soon apparent, I believe that it can restrict spontaneous (not enforced) activity also without impairing the appetite. Also, we certainly may not assume that feeding activity cannot be materially restricted at cyanide levels that have very little or no adverse effect on the efficiency of utilization of food for growth by fish in laboratory aquaria and on their consumption of food that can be procured with almost no effort. Although extremely low cyanide concentrations greatly impaired the swimming ability of young coho salmon, Oncorhynchus kisutch (Broderius, 1970), much higher concentrations not far below lethal levels were found, in a sinlgle experiment performed by Leduc (1966), to have no persistent, adverse effect on their food consumption and conversion efficiency to aquaria. Indeed, after an initial reduction of both food intake and food-coversion efficiency during the first 12 days of exposure the gross conversion efficiency considerably exceeded that of controls. This result needs verification, but there is no very good reason to doubt its validity. The efficiency of food conversion probably increased, as compared with that of the controls, because of reduction of the activity of the usually quite active fish in the cyanide solutions, in which more of the assimilated food consequently could be utilized for growth. Had the fish been required to remain normally active, a very different result probably would have been obtained. To ensure complete validity and comparability of laboratory measurements of food-conversion efficiency, uniform, moderate activity of all test subjects must be somehow enforced, but this is very difficult to accomplish. When this is not done, the results of detailed studies of food-conversion efficiency at a number of different levels of food intake and toxicant concentrations are certainly not without interest or value, but, for the reasons indicated, such a laborious study may not be quite as profitable an exercise as it may appear to be. I believe that effort devoted to feeding-activity studies can be more profitable, and that whenever an impairment of the efficiency of food utilization for growth is masked in aquarium tests by a depression of activity, the harmful effect of a poison will be revealed by appropriate tests for reduction of feeding activity. The activity of fish in the aquaria is largely spontaneous and unrelated to feeding, but feeding activity, which is not enforced activity, can be expected to be depressed by a poison whenever spontaneous activity is suppressed.

I have discussed in much detail the relation and distinction between appetite for food and feeding activity or foraging efficiency, and how they can be affected by water quality changes, because I believe that many biologists do not sufficiently realize the need for food-consumption studies designed to measure something other than the appetite or assimilative capacity. Very few studies of effects of water pollution on the foraging activity and success have been undertaken in the past. I believe that much effort can be profitably devoted to the development of methods for such investigation.

Sufficiently instructive tests for impairment of the feeding activity or efficiency of small fishes that feed on plankton or on benthic invertebrates such as amphipods in standing or gently flowing waters apparently can be quite simple, requiring little space and no elaborate facilities. One can introduce a limited number of the food organisms into each of several large aquaria and determine how many of them are consumed in a certain period of time by hungry fish that have been held in the aquaria for some time in the presence or in the absence of a poison. The number of food organisms introduced into each aquarium should be such as to make it impossible for the fish to become satiated. It can be less than the number that can be consumed by the fish at once when the food organisms are very abundant and easily found and caught, or it can be greater than that number if the initial density of the food organisms or the cover provided for them are such that these organisms are not too vulnerable to predation. The food organisms should be as uniform in size as possible. The food organisms remaining in the aquaria with and without the toxicant being tested can be counted when only a few remain in the control aquaria. If the foraging activity and efficiency of the fish are unaffected at a tested concentration of poison, about as many of the food organisms, on the average, should remain in the aquaria with the contaminated water as in the control aquaria at the end of the test period. Because of the progressive decline of the numbers of food organisms in the aquaria during a test, the experimental and control fish will be confronted with a desirable variety of foodorganism densities in such tests.

For experiments with large fish, small, artificial ponds like those that have been used in the already mentioned experiments on the influence of dissolved oxygen on the feeding and growth of largemouth bass can be used. However, such ponds are costly and require much space, and the maintenance in them of constant concentrations of toxic pollutants, by sufficiently rapid replacement of the water or otherwise, can be difficult. Therefore, experiments with laboratory models of more modest size have been undertaken recently. In these exploratory tests long, rectangular aquaria are being used, with a shelf made of fine-mesh wire or plastic netting suspended at each end a short distance below the water surface. Mosquitofish (*Gambusia*) with which these tanks are stocked soon learn to use the area over each shelf as a refuge, remaining there most of the time and escaping to one of these sanctuaries if they can when they are pursued by a largemouth bass also placed in the aquarium. The bass catch some of the mosquitofish that spontaneously leave the protection of the cover from time to time, or that the bass are able to flush from the cover by some maneuver, but they are unable to follow the prey in the shallow water above the shelves and capture it there. Consequently, they cannot fully satisfy their appetite, and their food consumption and growth are dependent on the density of the prey, just as were those of the bass in the ponds. Other things being constant, any reduction of their foraging vigor and agility must result in a reduction of food intake and slower growth.

Because of differences of the foods and feeding habits of fish of different kinds in various waters, experimental methods suitable for the study of effects of water pollution on the foraging activity and efficiency of some species are unsuited to other forms. The contriving of the most appropriate methods sometimes may not be easy, challenging the most imaginative and inventive biologist's ingenuity. But experiments that are very easily designed or standardized soon cease to be interesting. Artificial streams with circulated water can be used for tests with fishes that normally inhabit rapidly flowing waters.

In all such experiments it is most important to ensure that the activity and behavior of the food organisms (prey) will be unaffected by the pollutant at the concentration tested, or will be affected, at most, far less than the foraging activity of the test subjects is affected, if there is any effect. A debilitating effect on prey of the kind selected more serious than the effect on the predator can result in improvement of the predator's foraging efficiency under the experimental conditions. This improvement would not necessarily occur in nature, where important food organisms can be ones relatively resistant to the pollutant, and it certainly would conceal an actual reduction of the predator's activity. The food organisms selected should, therefore, be of a kind found through preliminary experiments to be relatively insensitive to the pollutant that is being tested, as compared with the fish that is the test subject. Fortunately, the mosquitofish, Gambusia affinis, and other related species with similar habits are handy fishes highly resistant to many poisons and highly suitable for use as prey in the experiments and in other respects. On the other hand, many of the predaceous species most valued by man as food and game fishes, especially those of the family Salmonidae, are relatively sensitive. Suitable food organisms to be preyed upon by small fishes usually should not be very difficult to find. Air-breathing aquatic insects such as mosquito larvae and pupae, which are not sensitive to many dissolved toxic substances, as well as to dissolved oxygen deficiency, and young of the hardy brine shrimp, Artemia salina, hatched in the laboratory from eggs that can be easily purchased, should not be overlooked in seeking suitable forms.

Since most poisons at low concentrations at which the appetite and food-conversion efficiency of fish used as test subjects are not materially affected can so affect the activity of resistant food organisms only after fairly long exposures, the duration of exposure of the food organisms to a poison usually should be minimal. They should be kept usually in clean water while the test subjects are being exposed to a toxicant in preparation for a foraging activity test. Small fish to be used as prey can be accustomed to the experimental environment and trained to avoid the predator by holding them for some time under the test conditions but in the absence of the poison until they are subjected to attack by fish previously exposed to the poison. However, if adverse effects of relatively high concentrations of poison are found to be produced very rapidly and subsequently to become less pronounced because of acclimation of the organisms to the poison, a different procedure may be advisable. All of the food organisms, including those to be fed to control fish, then can be acclimated before a test to the lower concentration to be tested. Various other ways to minimize effects of the tested impairment of water quality on the food organisms may be possible. For example, some clean water could be continuously introduced into experimental aquaria over the productive shelves described above that serve as cover for the prey (mosquitofish). The resulting, unavoidable dilution of the pollutant in the bulk of the aquarium water could be compensated for by continuous introduction of a sufficiently strong solution elsewhere in the tanks.

By the various experimental methods that have been discussed in some detail, a limit of water quality alteration not likely to have any considerable effect on the feeding and growth of the experimental subjects when the availability of food is constant can be determined. One can then proceed to the final step of the proposed scheme for the estimation of the limit of alteration having no material effect of any kind on the growth of the fish in their natural habitat. Possible effects on the food supply (production and availability of food organisms) next can be investigated experimentally with model environments, such as artificial or modified natural streams or ponds, in which foods are produced naturally and fish depend entirely on this natural production (except for some consumption of terrestrial organisms that may unavoidably enter into their diet). Experimental facilities and methods for such investigations cannot be adequately described here. Suitable methods that have been used at Oregon State University to study the effects of enrichment of water with sucrose on the foods and growths of trout in a modified natural trout stream have been described by Warren et al. (1964). Those used more recently in a similar study of effects of pulp and paper mill wastes in outdoor, artificial streams have been described briefly by Warren et al. (1974). Only the highest concentration of a toxic water pollutant that has been found not to affect fish directly so as to impair their feeding and growth may need to be tested in the difficult and costly experiments in which natural conditions must be reproduced as nearly as possible. This concentration may be found to have no demonstrable, adverse effect also on the food supply and growth of the fish in the simulated natural environment. However, if a considerable reduction of the growth of the fish is observed at this concentration, lower concentrations must be tested to determine the level at which there is no such effect and, therefore, no material effect on the availability and consumption of food.

In the foregoing discussion emphasis has been placed on toxic water pollutants, but effects of reduced concentrations of dissolved oxygen on the growth of fish also have been considered. The literature on the latter subject has been critically reviewed and the significance of the then available data thoroughly discussed by Doudoroff and Shumway (1970). In much the same way as oxygen deficiency, some toxic substances that interfere with external respiration may very markedly impair the appetite of fish but have little or no effect on their food-conversion efficiency when the fish receive uniformly restricted food rations. Because of the well-known influence of temperature on the metabolic rates of fish, thermal pollution, now of great importance, presents some special bioenergetic problems (Doudoroff, 1969) that can be only very briefly discussed here.

The temperature optimum for the growth of fish is a function of the food supply. At moderately elevated temperatures, fish may be able to grow much faster than they do at lower, normal temperatures when the food supply is unlimited, but more food is needed for mere maintenance of their body weight, because of the elevated metabolic rate. When the supply of food is limited so that the daily consumption cannot increase, growth is reduced as the temperature rises, not because of any impairment of, or interference with, metabolic processes, but only because of their acceleration and consequent reduction of the fraction of the energy of food that remains to be utilized for growth. Recent studies on salmonid fishes in aquaria and in artificial streams at Oregon State University have well demonstrated the importance of this effect. An increase of the activity of fish with rise of temperature can, of course, sometimes result in greatly increased exploitation of available food resources. However, much improvement of foraging efficiency may not be possible because of the nature of the food supply and of the feeding habits of the fish. The effect of a temperature increase on growth in the natural environment than can be just the opposite of that observed in laboratory tests when the food supply is unlimited. Since the gross efficiency of conversion of a limited amount of food is reduced at an elevated temperature at which the appetite for food is increased, there is superficial similarity between the thermal effects and those of poisons that impair metabolism while stimulating the appetite. Obviously, however, there are important physiological differences of these effects that should be recognized. Growth is more likely to be impaired markedly by a rise of temperature when uniformly restricted food supplies are small than when the daily rations are relatively large.

Although deposits of fat can be of great value to fish during periods of nutritional deficiency, mere deposition of fat should be distinguished from true growth, which is largely an increase of protein. Appropriate measurements of body composition should be made, therefore, in connection with studies of growth. Finally, I want to point out that low concentrations of some poisons may not only be harmless to fish in nature but also favor growth. There is some evidence that the vigor and foraging efficiency of fish, as well as their appetite, increase at low levels of some substances that generally are regarded as poisons only, so that growth may be promoted not only under artificial conditions. Such data should not be judged obviously erroneous.

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SECTION 7

MONITORING THE CONDITION OF FLOWING WATERS BY BIOLOGICAL ORGANISMS

Ruth Patrick

Whether one is concerned with the assimilative capacity of the river or its sport and commercial fisheries, one eventually has to be concerned with the whole ecosystem. In monitoring we may study one or a few selected species or we may assay the condition of the whole aquatic ecosystem in certain areas. It is important that natural streams be set apart and kept in their natural state, so we can have base lines against which we can measure natural change and changes due to man's effects.

In monitoring one may wish to learn about immediate change or more long-term, subtle changes. In such studies one must remember that time is a relative parameter. Organisms that reproduce once a day would show effects in two days that might take years to show in an organism that reproduces much more slowly. One type of monitoring the condition of organisms is by bioassay tests. The time and duration of the test to show acute or subacute effects depend considerably on the organism under study. However, generally we use bioassay tests of a few hours or a few days to determine acute effects--that is, those effects that show up immediately. In the United States these short-term tests are designed to determine the concentration at which 50 percent of the organisms die in a given length of time. They are, if batch tests, often considered as more-or-less "rough and ready" tests to get an idea of the effects of a given substance on aquatic organisms in an ecosystem. In carrying out such tests it is advisable to use organisms representing various stages of the food web, because the food web may be altered if any stage of nutrient and energy transfer is impaired. For this reason an alga that is a good source of food, an invertebrate, and a fish are often tested.

Long-term monitoring tests are aimed at showing sublethal effects and often follow acute tests, because by the acute tests one has found that concentration or range of concentrations that probably will not kill the organism being studied. Whereas acute tests are concerned with concentrations that cause death; sudden morphological changes such as the sloughing of mucus by fish; or avoidance reactions to low oxygen; long-term tests are more concerned with physiological changes and changes relating to the fecundity of the organism.

In such tests one may examine histological changes in gills, liver, and pancreas; physiological changes in respiration rates; or enzymatic changes. For example, Hinton, et al. (1973) have found that DDT inhibits the formation of ATP--that is, adenosine triphosphatase. Other enzymes often studied as to the effects of a given waste are d-hydrogenase, acid phosphatase, and carbonic anhydrase.

In such long-term or chronic tests one often measures the build-up or accumulation of materials within the cells such as heavy metals, radioactive materials, or some of the chlorinated hydrocarbons. Behavioral tests are often used in these long-term chronic tests. For example, Cairns and Scheier (1964) found the dieldrin will interfere with the sight of certain fish at extremely low concentrations, thus they are not able to see their food as well nor, in the case of schooling fish, are they able to school. One also is concerned about the effect upon the fecundity of organisms. Shifts in temperature or chemicals that alter the food species may affect the fecundity of the female and success of offspring. If the fecundity of a species is changed very much it may alter the whole food web.

Recent work of Patrick et al. (1975) has shown that minute amounts of heavy metals such as nickel, vanadium, and chromium may alter the species composition of the algae in a community and thus greatly change the plant food source of the food web. If such changes occur and the primary production is carried out by species of low food value, the productivity of the rest of the food chain will be greatly reduced.

Monitoring may be concerned with changes in individual species in the waters in which they live or with changes in communities. If one is studying one or a few species, such studies are usually carried out by isolating the species under study either in the field or in semi natural conditions. For example, oysters or clams are often sorted as to size and age class and placed into large trays with each oyster or clam being marked; thus over time one can study growth, the attack by disease, and the condition of the oyster or clam in question by sacrificing the individual organism (Figures 1, 2). One can also determine the accumulation of heavy metals, or radioactive materials, or of chlorinated or polycyclic hydrocarbons. Thus such studies are valuable not only to monitor the potential of the commercial crop in the area but also to monitor whether or not certain toxicants have passed through the estuary.

Fish in aquaria are sometimes used to monitor the effect of a given waste as it is being discharged. Cairns et al. (1973) have described such a methodology. Fish can be sacrificed from time to time to discover histological and physiological changes as well as the observations from day to day of death. In such studies of fish or oysters the same organisms may be studied over time and thus the monitoring is continual, although observation may not be continual. More recently, Dr. Burton of the Academy laboratories has developed methods of inserting probes into crabs and thus being able to continually monitor the heartbeat and various kinds of biochemical changes within the crab.

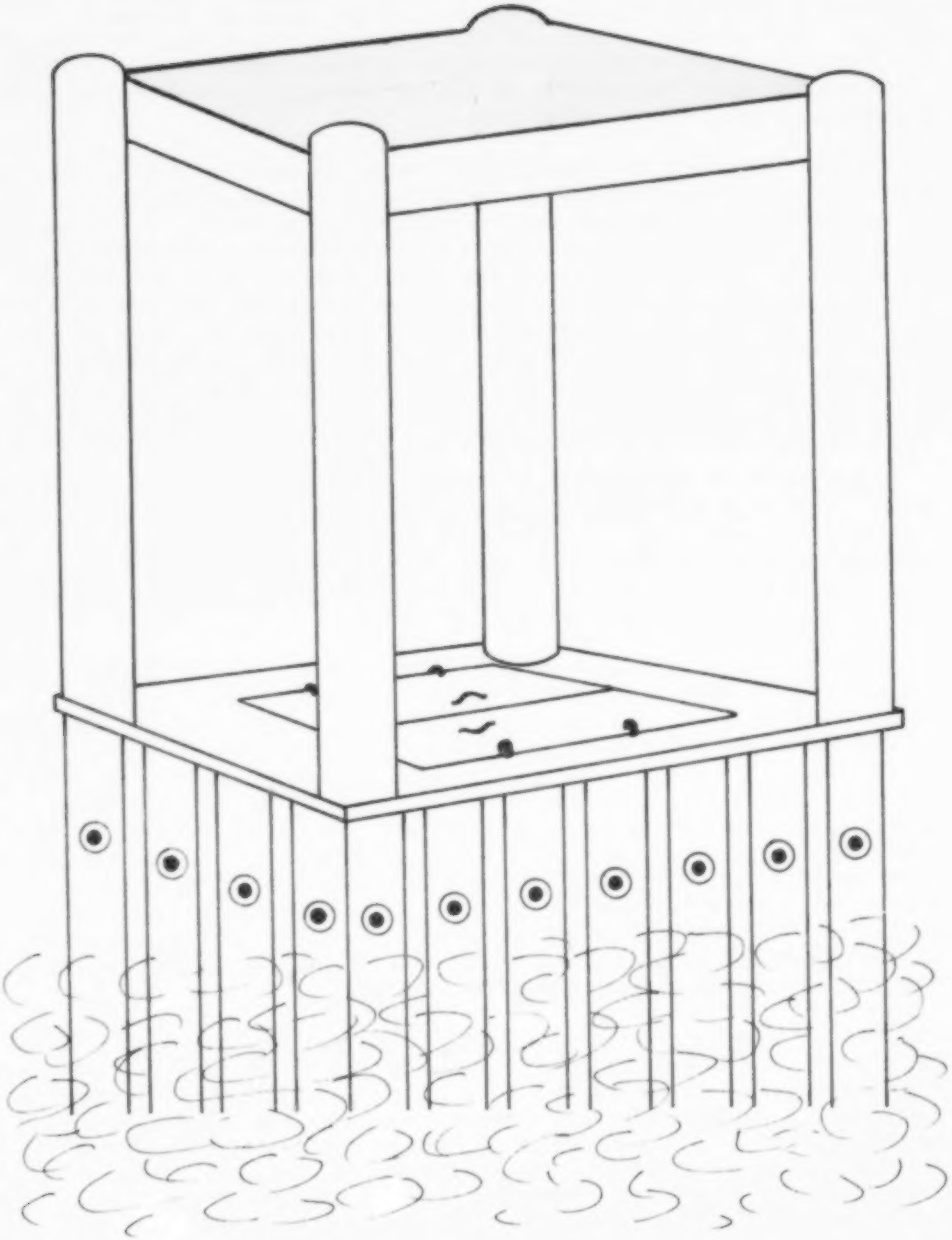

Figure 1. Platform from which oyster trays are suspended.

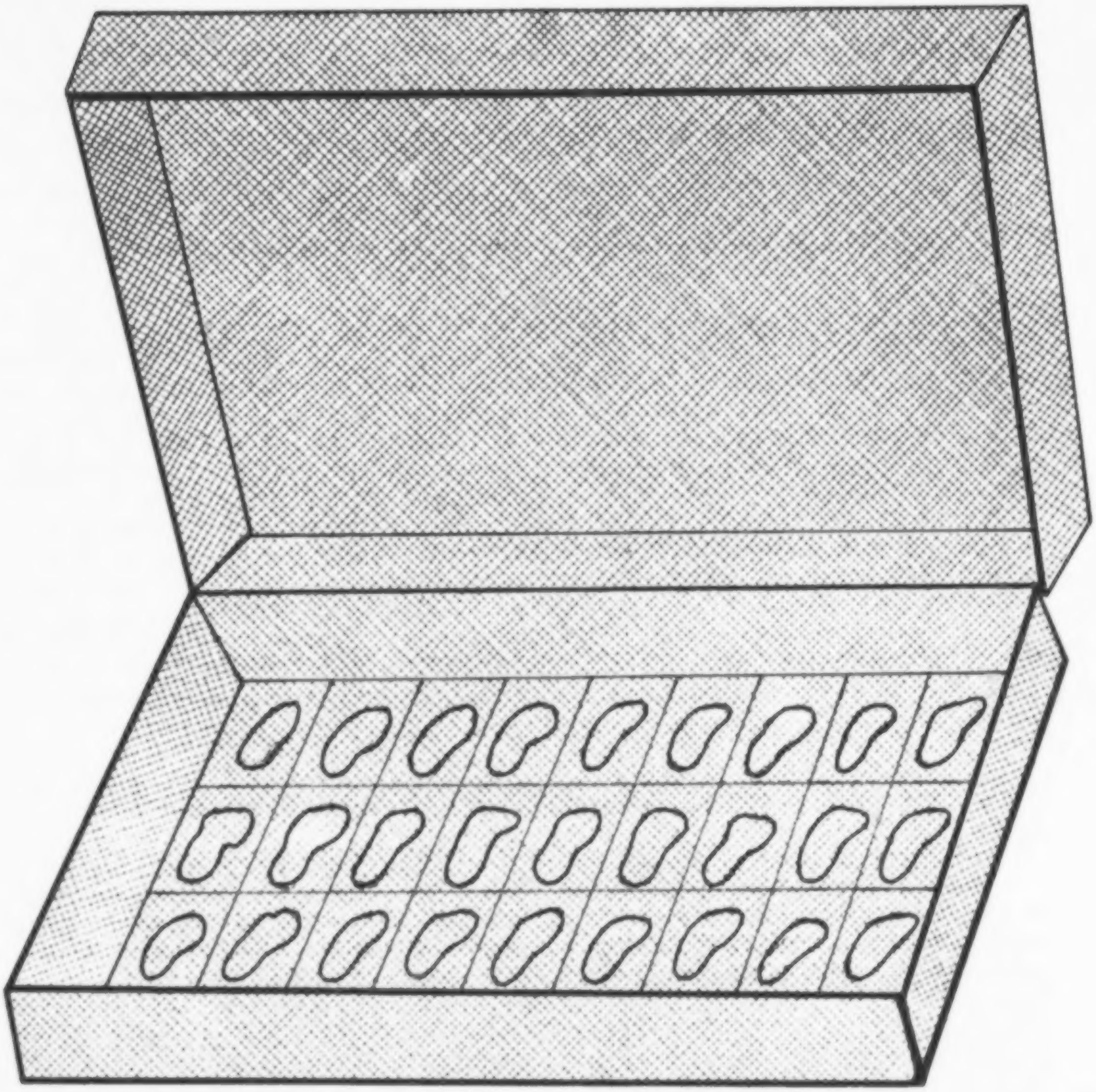

Figure 2. Oyster tray.

Sometimes a given species such as oysters in an oyster bed is intermittently monitored by taking grab samples; however, if this methodology is used one must very carefully design the experiment so that the total number of grabs will give reproducible results--in other words, that there will be given degree of statistical reliability that if the procedure is repeated the same kind of data will be obtained if no change occurs. This type of monitoring has been developed by the laboratories of the Academy of Natural Sciences (Patrick, in press).

Another type of monitoring is that which has been developed for monitoring communities of organisms. The most sophisticated of these has been those developed by Patrick et al. (1954) for algal communities growing on glass slides. In this method an apparatus known as a diatometer is introduced into a body of water. It has been found that diatoms grow successfully on these slides. This fact was first pointed out by Butcher (1947). However, the method used by Patrick is the first one to model the community and to note by changes in the structure of the community as well as in the kinds of species the effects of a pollutant. For example, it has been found that under natural conditions the structure of the diatom community conforms to a truncated normal curve (Figure 3) and that this curve remains fairly constant over time (Table 1). However, if pollution high in nutrients is introduced such as those high in nitrogen, phosphorus, and carbon, certain species will become extremely common and produce a long tail to the curve (Figure 4). Under toxic conditions typically one finds a reduction in numbers of species and the sizes of populations, although in some cases a few species that can withstand or tolerate the toxicant become very common because there is little competition by other species for the nutrients in the system and predator pressure has been greatly reduced (Figure 5).

By this diatometer method of studying algal communities one cannot only study shifts in the diatom community, but can determine whether or not shifts from diatoms to other species are occurring.

These diatometers can also be inserted into various reaches of a river to determine the relative degrees of eutrophication of the areas by the total biomass and kinds of species produced on the slides. Thus they are valuable in regional studies of eutrophication. In some instances they have been found extremely useful in determining the presence of small amounts of heavy metals or radioactive materials because some metals are concentrated by the algae to amounts many thousands of times the concentration of the ambient medium. Algae growing on these slides can also be used in determining primary productivity and P/R ratios of algal communities. We have found that these measures are important in determining small or sublethal shifts in the community. Likewise, one can extract pigments from them and determine a shift in pigment concentrations.

Periphyton are particularly good organisms to study because they have short life cycles and often produce chronic effects due to low amounts of toxicants much more rapidly than many larger macroinvertebrates. Furthermore, we know a considerable amount about the kinds of species and what they indicate. For example, the diatoms Nitzschia palea and Gomphonema

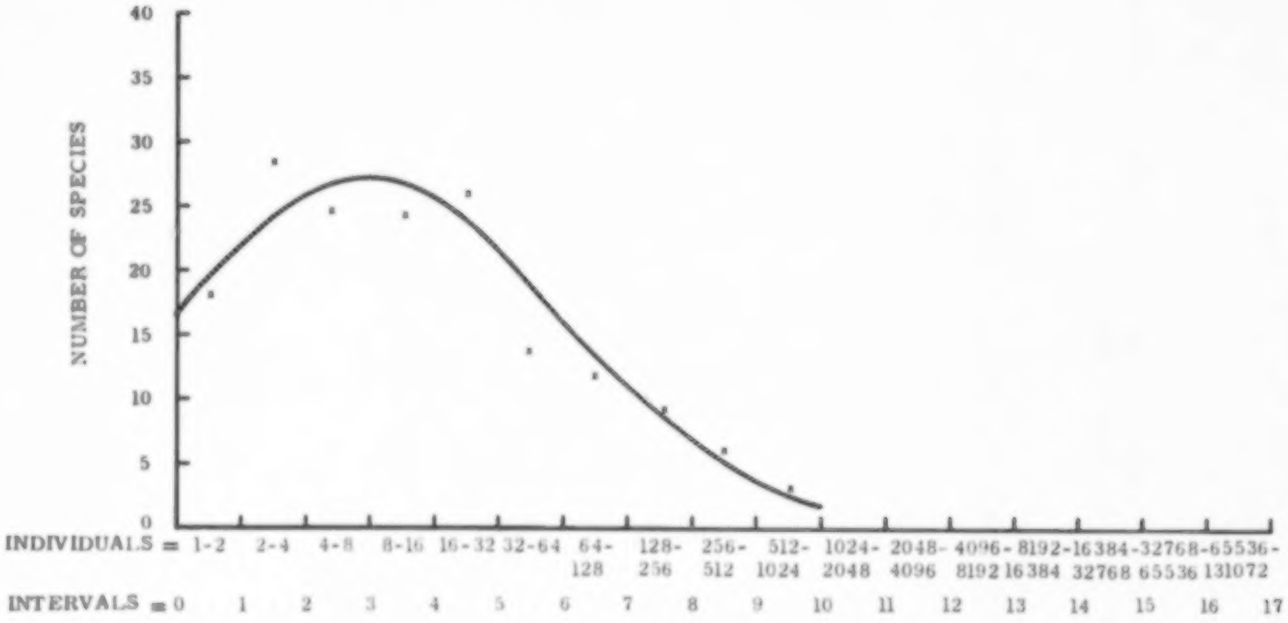

Figure 3. The structure of a natural diatom community.

TABLE 1. SUMMARY OF CATHERWOOD DIATOMETER READINGS AT STATION 1 OCTOBER 1953 TO JANUARY 1958

Date	Specimen number of modal interval	Species in mode	Species observed	Species in theoretical universe
Oct. 1953	4-8	22	150	178
Jan. 1954	4-8	19	151	181
Apr. 1954	2-4	24	169	200
July 1954	2-4	23	153	193
Oct. 1954	4-8	21	142	168
Jan. 1955	4-8	19	132	166
Apr. 1955	2-4	25	165	221
July 1955	2-4	20	132	180
Oct. 1955	2-4	27	171	253
Jan. 1956	2-4	30	185	229
Apr. 1956	4-8	35	215	252
July 1956	2-4	24	147	185
Oct. 1956	2-4	23	149	206
Jan. 1957	2-4	29	177	233
Apr. 1957	2-4	21	132	185
July 1957	4-8	29	181	203
Oct. 1957	2-4	25	157	232
Jan. 1958	2-4	27	152	212
(Apr. 1954-1958 averages)		24	151	194

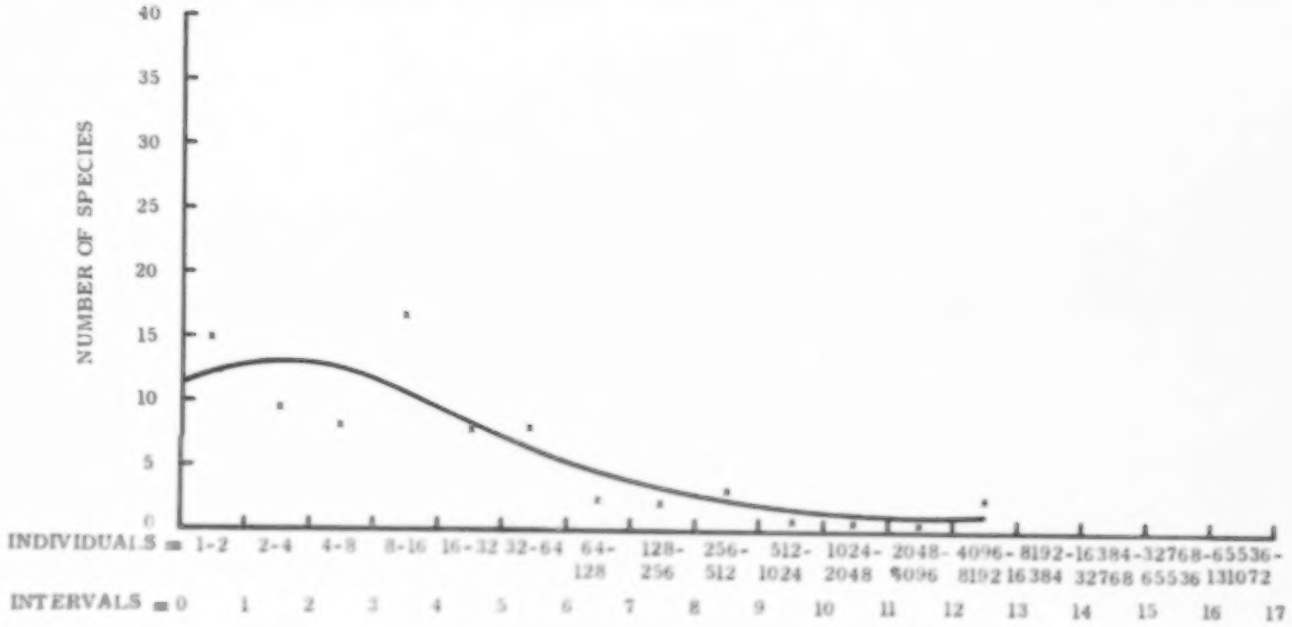

Figure 4. The structure of a diatom community under the effects of pollution high in nutrients.

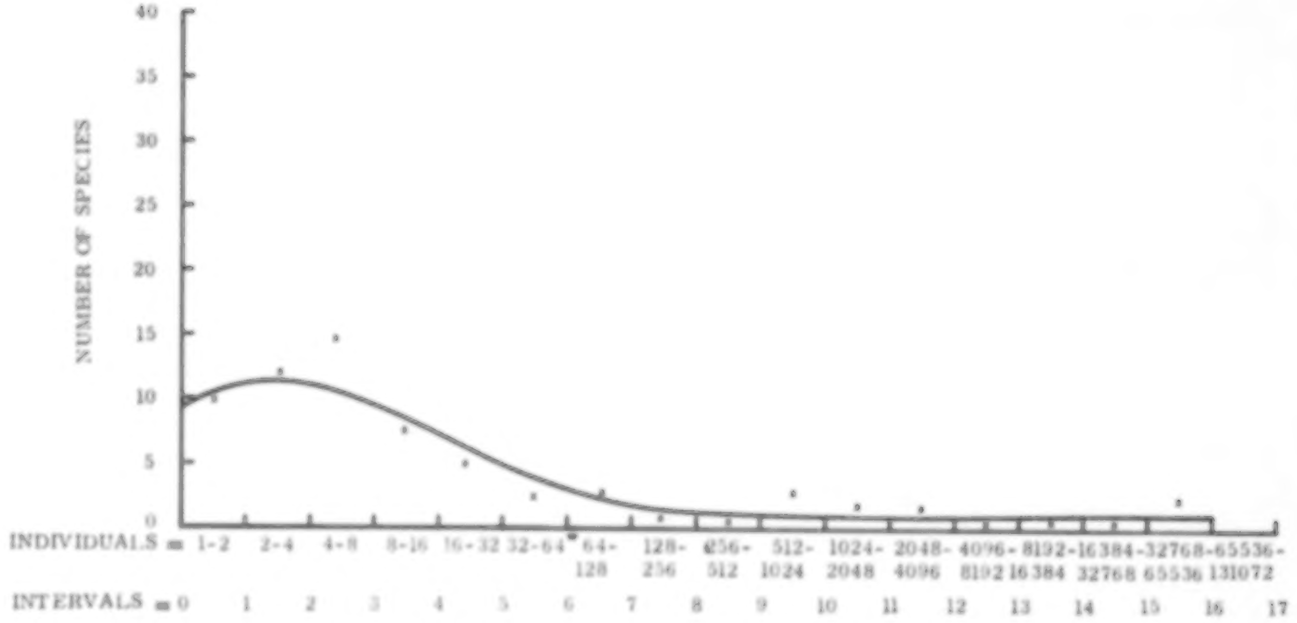

Figure 5. The structure of a diatom community under the effects of toxic conditions.

parvulum typically develop large populations under nutrient-rich conditions. The presence in abundance of Cyclotella meneghiniana in contrast to Cyclotella stelligera and C. kutzingiana indicates an increase in the nutrient levels of the water. There are a great many diatoms that indicate these shifts. Studies by Patrick (1956) have shown that diatoms have a toxicity threshold similar to that of fish and invertebrates to many industrial wastes, and thus a very short-term test can tell a considerable amount about the effects of a toxicant in a body of water on other members of the food web.

Various types of substrates have also been developed for monitoring invertebrate communities. Whereas the diatometer substrates (Patrick et al., 1954) have been devised to reliably represent the community in the river under study, this has not been done so far as I know for invertebrates. However, various people have studied how many invertebrate samplers one needs in an area in order to get reproducible results (Beak, et al., 1973).

One type of substrate that has been used are panels which collect sessile organisms (Figure 6). This is a substrate sampler consisting of a series of flat substrates placed in the water. This has been found to be very good for the collection of certain invertebrates. Other people have used various kinds of baskets from simple chicken-wire baskets filled with rotted wood to baskets of very definite structure such as barbeque baskets. These have been found very useful, but the important thing is to calibrate them so that one can obtain reproducible results and know the extent these organisms represent the area under study. In this way one can compare over time changes in a given area and the shifts between areas.

In the macroinvertebrate studies, as in periphyton or diatom studies, one is concerned about shifts in the dominant forms or shifts in the sizes of populations; shifts in kinds of species; and shifts in numbers of species. For example, by insect traps placed by Dr. Roback of the Academy in the Savannah River he was able to clearly define the effects of dredging, because the filter-feeders such as caddisflies disappeared as long as the water had a high suspended solids load. One can also determine different degrees of nutrient loading in the water by shifts in the faunas of insects such as shifts in a mayfly-stonefly dominated fauna to one dominated by damselflies and dragonflies, to one dominated by chironomids and worms. Since mayflies and stoneflies are particularly sensitive to oxygen concentrations, the loss of these species indicates an oxygen sag at times in the river even though it is not noted chemically. In other cases shifts of Hydropsyche caddisflies to dragonflies and Chematopsyche caddisflies have indicated intermittent toxicity.

A second type of monitoring is one in which given selected areas of a river are studied over time. This type of monitoring is extremely valuable because it tells a great deal more than monitoring by means of substrates. In such monitoring a team of scientists is sent into a river. They determine not only changes in the chemical and physical characteristics but also the characteristics of the aquatic ecosystems. They are able to determine by increased growth of various types of organisms such as submerged or emer-

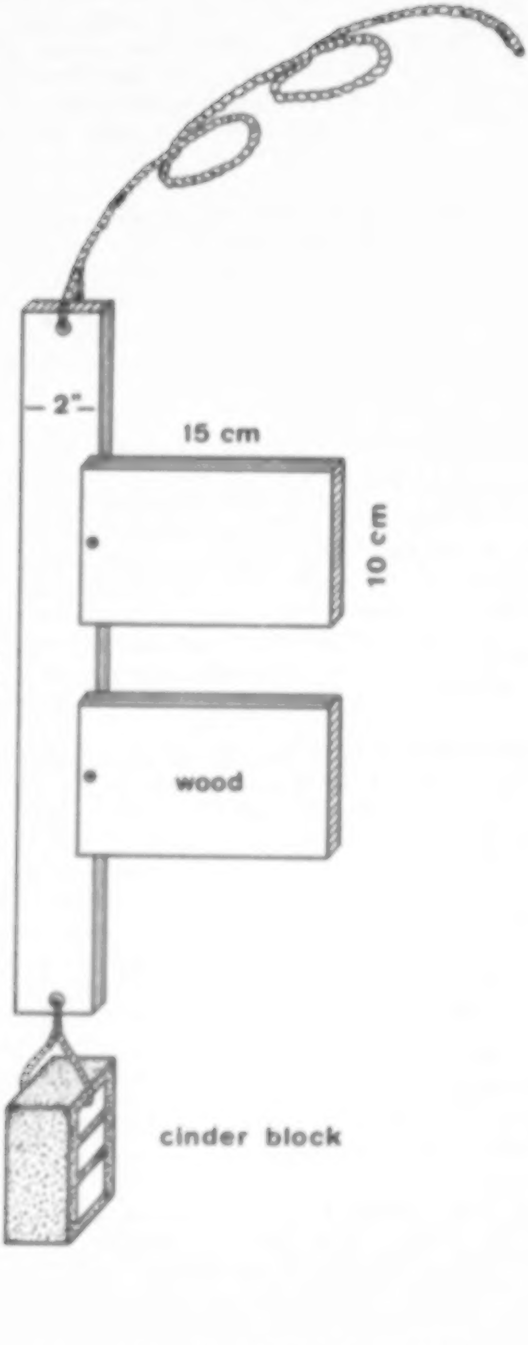
2"
15 cm
10 cm
18"
wood
cinder block

Figure 6. Invertebrate sampler.

gent aquatic plants if the nutrient content of the water and sediments are high. Beds of Sphaerotilus indicate a significant increase in carbonaceaous materials in the water. Furthermore, such studies are able to determine shifts in the diversity of habitats or physical changes in the river channel. This information is not determined by monitoring with substrates, for by the use of substrates one simply determines facts concerning the organisms, but not the condition of the area in which they live.

Another difference is that when one sends a team of scientists into a body of water one determines facts concerning many different groups of organisms. This is extremely important if one wants to determine subtle changes. Often an ecological change that has nothing to do with pollution will affect a single group of organisms, but it is extremely rare that an ecological change will affect many groups of organisms such as the algae; invertebrates such as molluscs and worms; insects; and fish. This methodology was first developed by my staff at the Academy of Natural Sciences in 1948. Therefore, the more lines of evidence from different kinds of organisms that one has the more sure one is of his diagnosis of conditions. Such studies are particularly valuable when one is concerned with small sublethal effects which are important to detect before they become problems. Wth the possible problems in our country due to increases in chlorinated hydrocarbons, heavy metals, and radioactivity, this type of thorough examination of conditions in monitoring becomes more important. Of course, this type of monitoring is intermittent, and it is more expensive, and therefore between studies things may occur that one does not realize. For this reason it is best to combine a continuous monitoring system with this more thorough, intermittent system. I often compare these types of studies to medical treatment. If one wants to know if something is wrong a simple procedure can be used such as examining the condition of a single group of organisms. This compares with taking one's temperature or doing a cardiogram. But if one wants to understand trends or causes of change a thorough study of aquatic areas or a detailed physical examination of an individual is needed.

As noted above, the kinds of changes which one observes are first the changes in relative sizes of populations of species. If we find that those species that are tolerant to a given type of pollution are becoming more common, then one strongly suspects that it is present. For example, in a stream in eastern United States if one finds a shift from mayflies and stoneflies and certain species of caddisflies being very common to a great increase in chironomids, dragonflies, Physa snails, the limpid, Ferrissia, and tubificid worms, we know that the nutrient load of the river has increased. Furthermore, if we find that only organisms such as the flatworm Dugesia tigrina, Ferrissia tarda, Physa heterostropha, and tubificid worms are present we know that the degradation is caused by increased nutrients and resultant increase in bacteria, and probably no toxicity. However, if we find and increase in certain of the chironomids and of certain dragonfly larvae without an increase in the above mentioned species, we can infer that the organic load may occasionally have low levels of toxicity present (Patrick observations).

Temperature is another common pollutant that is often difficult to detect unless continual monitoring is carried out, and then it is sometimes difficult to predict the temperature regime in the river. However, shifts in algal species will clearly denote these kinds of changes. For example, we have found that if the temperature of the water consistently remains below 30 C and no other pollutant is prevalent, diatoms will be dominant throughout the year in most streams, particularly in eastern United States. If the temperature during the summer increases to between 30 C and 33 C green algae will predominate, and some blue-green algae will become very common. Thus one can estimate the temperature regimes in various parts of a body of water.

Another type of determining change, particularly in lakes, has been to examine the fossil record. In such studies sediment cores are taken and dated. The shift in diatoms and invertebrates species enables one to determine trends toward eutrophication.

From this discussion it is evident that the monitoring of biological organisms can be very valuable in determining the effects of wastes. As contrasted with chemical and physical determinations of water quality, the organisms integrate over time all deleterious effects, whereas a chemical examination only determines the presence of the chemical for which analysis is made at the particular time. Actually it is important that both types of studies be made. The biological studies often give an indication of a certain type of chemical or deleterious conditions being present. It is then necessary to determine exactly what chemical is causing the effect. Therefore, both types of studies become important, but the biological studies are the better continual monitoring studies if only one type of monitoring is to be made, because it integrates all changes which may occur.

In the United States we are also realizing the importance of preserving specimens from monitoring studies. Recently there was a considerable scare about the accumulation of mercury in fish. However, an examination of fish in our museum collections showed that these older specimens had similar amounts of mercury and the sudden awareness of the presence of mercury was due to better analytical techniques. Without these specimens in our museums such comparisons would not have been possible.

From these various examples it is evident that biological monitoring can be useful to determine the extent and degree of harm in an area of a specific waste. Diatometers and similar samplers can be very useful in determining trends over long reaches in a river system of increases in pollution. Organisms such as diatoms and some invertebrates can pick up and concentrate over time amounts of chemicals infrequently discharged that would probably not be picked up by ordinary chemical monitoring. Continuous monitoring as well as intermittent monitoring is extremely useful in comparing changes over long periods of time in various bodies of water.

The program of monitoring depends on the questions one wishes to ask, particularly whether one wants to determine general changes or whether one wants to determine trends or more precise causes of change.

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SECTION 8

THE ROLE OF ALGAE IN THE POLLUTION OF RESERVOIRS AND PROBLEMS OF CONTROLLING THEIR NUMBERS

V.G. Khobot'ev[1]

Water pollution, both marine and freshwater, from industrial wastes changes the living conditions for all living organisms and disturbs the established communities. In some polluted waters, with nutrient enrichment and slightly increased temperature, favorable conditions are created for massive development of algae. Algal cell counts reach millions or even billions in a single liter.

The massive development of phytoplankton creates a considerable nuisance in water supplies, since it often disturbs treatment processes and impairs the quality of water produced. Algal blooms in water bodies of this type promote the intensified growth in underground mains and equipment, complicating treatment and sometimes causing equipment failure.

In cooling ponds blooms facilitate the formation of thick, compact surface films that hinder evaporation and heat loss from the surface, thereby reducing normal cooling of waste water. The growth of algae also reduced CO_2 through intense photosynthesis and produces scum on the inner surfaces of heat-exchange equipment. Removal of this scum frequently requires a considerable expenditure of labor, time, and resources. Blooms caused by blue-green algae also considerably degrade drinking water quality, giving the water an unpleasant taste and odor. The taste is caused by the emission of sulphur-containing compounds produced by the blue-green algae. Methymercaptan, dimethylmercaptan, isopropylmercaptan, dimethylsulphide, and others have been identified in decaying cultures of these algae. The odor of dimethylsulphide, brought about by the presence of such amines as methylamine and ethylamine, strongly resembles the smell of fish. A similar smell in natural water associated with the development of certain species of algae is caused by dimethylsulphide.

The massive development of algae creates difficulties in the operation of other water plants as well as in canals and irrigation systems. For control of phytoplankton, different means have been widely and effectively used in many cases. Biological, physical, mechanical and chemical methods of controlling "blooms" in reservoirs are well-known.

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[1] Moscow State University

The biological method uses biological filtration. One method is that proposed by S.N. Skadovskiy--a method in which a cascade arrangement of selected aquatic organisms in the water purifies it. In the upper portion of the cascade large sections of the bottom are populated with filter feeders such as freshwater mollusks (Unionids and Anodonts), which are capable of filtering up to 2000 liters of water per day at a distribution density of up to 70 individuals per square meter. These are followed by water plants, which reduce the dissolved nitrogen in the water to a minimum. Biological communities growing on surfaces as overgrowths will further reduce the nuisance organisms. Such communities reduce the number of phytoplankton cells by 60-70%, saprophytic bacteria by 70-80% and intestinal bacilli by 30-50%.

The mechanical method is based on various filter systems through which the water is filtered and suspended particles are removed. During heavy bloom, however, the filters quickly plug and they must be cleaned, sometimes every 20-30 minutes. The efficiency in such filters fluctuates from 20-60%.

The physical method of combatting bloom is based on the destruction of algal cells using ultrasonics or an electric current. The shortcoming of this method is the need for additional equipment to remove the slimy mass that is obtained.

The chemical method is most used for preventing blooms. To date, hundreds of chemical compounds have been tested as algicides to suppress the development of algae. Copper sulphate and chlorine are utilized most frequently. Their effectiveness, however, depends on the acidity of the environment. The copper ion in copper sulphate is toxic for algae, therefore, the effect of this ion depends on the concentration of hydrogen ions, i.e., the lower the pH, the more toxic the ion becomes. Since pH is greater than 7 in the majority of water bodies, the effectiveness of this agent is considerably reduced. Active complexing of copper ions with ligands in industrial water supplies also often leads to a reduction in the toxicity of copper sulphate.

The chlorine in hypochlorous acid is toxic. Because of its strong oxidizing efect, chlorine penetrates into plant cells and damages vital centers. Chlorine concentration also depends on the pH of the solution and it increases only with a reduced pH.

Besides pH, the selection of algicides depends on the capacity of the algae to adapt to the effect of the preparations. If only 2-3 species of the 50-60 algae species encountered in a water body adapt, then difficulties can occur for the industrial utilization of the water since these species can cause a bloom and occur in huge numbers. The selection of different chemical substances which are toxic to these specific organisms can be the best solution. A collection of 2-3 algicides provides the possibility of completely suppressing blooms in the water. However, in selecting algicides, one must consider the features of the water body being treated. The substance should possess toxicity with regard to the greatest number of algal species, have the capacity to penetrate easily into vitally important centers of the organism, not react with chemical compounds contained in the water and be available for use. In addition, the algicides should be safe for man, harmless to fish and non-corrosive to metallic parts of equipment. Chemicals such as 2,3-dichloronaphthoquinone (also called figone, frigone, 2,3DNA, 2,3SNA) and hexachlorobutadiene are suggested as algicides, as well as rosein-amide-D-acetate, monuron, simazine and many other algicides.

The physiological effect of some algicides, monuron and diuron on blue-green algae in particular, amounts to an acute inhibition of photosynthesis. Diuron, the molecules of which contain two chlorine atoms and methyl groups in addition to the phenol ring, possess the most clearly-pronounced effect on blue-green algae. However, the introduction of these algicides into a reservoir impairs the organoleptic properties of the water, and the processes of nitri- and nitrofication are disturbed. Through toxicity tests on other aquatic organisms 2 and 10 mg/liter were shown to have a substantial effect on blood morphology, phagocytic activity of leucocytes and other changes in test animals.

Many algicides are volatile or readily hydrolyzed, thereby requiring repeated application, sometimes 3-4 times in a summer.

Searchers for new compounds to effectively protect the water from blooms led us to study the effect of complex ores and products of their processing on various species of algae. Together with A.P. Terent'ev and N.S. Stroganov, we established that complex ores containing zinc, copper, cadmium, nickel, lead, silver, and other chemical elements act effectively on the algae which produce blooms in water bodies. In tests 2 mg/liter reduced the number of algae cells in 30 days and eliminated them in 45 days. Under the effect of complex ores, the filamentous alga, Cladophora, decreased sharply in biomass and the colony darkened and decomposed into separate cells. Green algae proved more resistant to the effect of ores, but was severely depressed. First the chlorophyll disappears, then the cells lose pigment and in 30 days they are almost entirely dead.

The effect of complex ores is a complex process, but the investigations conducted show that the slow change of complex metals into a soluble state and their low initial concentration evidently favor their inclusion into the algae's biochemical reactions and lead to inhibition of various vital functions. The slow and weak dissolution of the ores is easily overcome if the ores are introduced into the water body 10-15 days prior to the beginning of phytoplankton development. In some cases, it is convenient to introduce the algicide into the ice in the spring so that water contains a solution of substances from the complex ore in the required concentration prior to the spring outbreak of algae development. Thus, one can prevent an increase in the number of algae by controlling their numbers, and not just decreasing them. Complex ores, usable as algicides, are resistant to hydrolysis and not susceptible to destruction by bacteria. Their slow dissolution in water makes it possible to maintain a concentration in the reservoir which is toxic for algae for a long period of time.

For the purpose of controlling the quantity of plankton organisms in industrial water supplies, we tested another class--organostannic compounds and trialkyl/aryl/substituted compounds in particular, which possess fungicidal and insecticidal properties. We tested trimethylstannanol and trimethylacetoxy-stannane which strongly suppressed the vital functions of algae even at a concentration of 0.2 mg/liter. In the same concentrations, these compounds suppressed the reproduction process in representatives of zooplankton. The advantage of organostannic compounds over other algicides is that they are toxic for plankton organisms at considereably lower concentrations.

However, it is important to use substances that are selectively toxic to the undesirable algae. Often blooms must be suppressed without harming the development of zooplankton and other aquatic organisms. Phytoplankton toxicity tests with silicone compounds (vinyl-triethoxysilane, tetraethoxysilane, trifluoropropinyldiethoxysilane, and others) showed that 0.01 mg/liter depresses the number of algae by roughly 94-96% in the first days of the test; however, in the subsequent 15 days the number of cells increases slightly and reaches 50% of the control. Tests on Daphnia and Mollusca showed 100% survival even at 1 mg/liter of silicone compounds. The number of young borne by Daphnia in the test proved to be greater than in the control.

By comparison of the sensitivity of representatives of zoo- and phytoplankton to silicone compounds, the high selectivity of phytoplankton is revealed, as well as low sensitivity or stimulation of Daphnia. The ability of silicone compounds to reduce algae cells can be utilized to suppress the development of phytoplankton during blooms.

Alkoxysilanes have the advantage over previously-used algicides to combat blooms because they are comparatively quickly destroyed in water and have a selective effect on phytoplankton, while not suppressing the vital activity of Daphnia and Mollusca.

The development and utilization of toxic substances, which possess a narrow selective effect, opens a path towards the synthesis of substances with prescribed toxicity for certain aquatic organisms and is the basis for the development of the best methods of controlling the number of zoo- and phytoplankton in reservoirs.

No one method is best and there should be several such methods for specific purposes for each water body.

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SECTION 9

EUTROPHICATION IN THE UNITED STATES: PAST-PRESENT-FUTURE

A.F. Bartsch, K.W. Malueg, C.F. Powers, and T.E. Maloney

Chief Seattle, leader of the Suquamish tribe in the Washington Territory, delivered a prophetic speech in 1854. The occasion was to mark the transferral of ancestral Indian lands to the federal government. His words indicate much greater understanding of man's position in the natural system than we seem to have today, for he stated:

> This shining water that moves in the streams and rivers is not just water but the blood of our ancestors. If we sell you land, you must remember that it is sacred, and that each ghostly reflection in the clear water of the lakes tells of events and memories in the life of my people. The water's murmur is the voice of my father's father.
>
> The rivers are our brothers, they quench our thirst. The rivers carry our canoes, and feed our children. If we sell you our land you must remember, and teach your children, that the rivers are our brothers, and yours, and you must henceforth give the rivers the kindness you would give any brother.
>
> ...The earth does not belong to man; man belongs to the earth.

In the United States we have failed for too long to teach our children that the waters are sacred. Our rivers still carry our canoes, our boats, our ships, but very few of them could feed our children. Most of our lakes are not clear. Many offer no lovely reflection to a potentially admiring glance because most of the time, they are covered with algae or higher aquatic plants.

As elsewhere throughout the world, eutrophication is now a familiar problem in the United States. This progressive nutrient enrichment of lakes and their responding increased biological production with its related consequences are the major threat to many lakes. Lakes typically evolve from a state of low productivity and relative high purity to one of increased productivity and lessened quality. This process often is marked by nuisance algae or other plant growths, drastically reduced oxygen content in the deeper waters, and bad tastes and odors. Reaching this stage usually is a lengthy process, sometimes even requiring thousands of years.

However, when a lake is subjected to heavy pollution and other forms of human population pressure, eutrophication proceeds much more rapidly. This accelerated process has been termed "cultural" eutrophication.

THE PAST (1850-1967)

Cultural eutrophication was recognized as a problem in this country at least as early as 1850 when complaints about unpleasant odors from Lake Monona that assailed the citizens at Madison, Wisconsin, were published in local newspapers. Eutrophication research in this country appears to have started in the early 1900's with the work of Birge and Juday at the University of Wisconsin. Because eutrophication is fundamentally an expression of the metabolism of lakes, studies of limnology and eutrophication go hand in hand.

In these early years limnology centered around the taxonomy, and to a lesser degree, the ecology of the zooplankton and around descriptive investigations of lake phenomena. Included particularly were the areal and seasonal distributions of temperature, dissolved gases, and solar radiation. Increased interest in the chemistry of lake waters developed during the second quarter-century. There was special interest in nutrients, pH, Eh, organic matter, and oxygen consumed, all parameters related directly to lake productivity and trophic state, and hence eutrophication.

In the 1930's and 1940's more attention was given to cycling of nutrients in lakes. The awareness, for example, that algal populations could be maintained or increased with no apparent changes in lake-water concentrations of available phosphorus, or in the presence of no detectable available phosphorus (or nitrogen), led to renewed investigations in these areas. It then became evident that in many lakes the nutrient elements were undergoing very rapid cyclical changes, moving between bottom sediments and overlying water, from dead organic matter to the water, from the water to actively photosynthesizing plants and to bacteria. These precepts are fundamental to an understanding of the eutrophication process and are basic to the concept of limiting nutrients.

Following World War II increased attention was devoted to the accelerated eutrophication of lakes by nutrients from cultural sources. For example, experience with the Madison, Wisconsin, lakes, Lake Washington at Seattle, and a number of European lakes (particularly Zurichsee) made it increasingly evident that nutrients introduced by numerous activities of man--from both point and non-point sources--could lead to rapid and serious deterioration of water quality. As a consequences, a number of remedial approaches were taken to curb nutrient contributions to lakes. At Madison, where municipal sewage had long been discharged into the chain of lakes, the following diversions took place: (a) from Lake Mendota in 1899, (b) from Lake Monona in 1936, and (c) from Lake Waubesa in 1958. At Seattle's Lake Washington, effluents from 11 treatment plants were diverted to Puget Sound between 1963 and 1968. After the first diversion the lake's condition began to improve and has continued to do so. The abundance of phytoplankton has decreased. Secchi disc transparency, which had fallen from 3.7 in 1950 to 1.0 in 1963, returned to 3.5 m in 1971. The lake is now no longer considered eutrophic by many investigators.

In the 1950's sufficient evidence had already come to hand to strongly suggest that even huge bodies of fresh water such as the Great Lakes are not immune to cultural eutrophication. Lake Erie, the shallowest and most polluted of the five, was the first to exhibit serious deterioration. Subsequently, similar changes in lake water quality were detected in southern Lake Michigan, Lake Ontario, and other areas of the Great Lakes. Although these lakes had been studied for many years from the point of view of fisheries management, very little limnological work had been done prior to the fifties. These evident eutrophication trends, however, served to stimulated greatly increased research efforts at a number of United States and Canadian universities, and by the governments of the two countries as well. Great strides have since been made in furthering the knowledge of these important lakes, leading to more intelligent management and utilization of the resource.

Also in the 1950's, and indeed as far back as the 1940's, emphasis began to shift from descriptive to experimental limnology, in which manipulations are carried out on the full-scale or pilot-scale level, utilizing lakes, ponds, or physical models. Such experimental methodology has become fundamental to the development of lake-restoration techniques and procedures, wherein results from the laboratory are carried to the field for testing under controlled conditions.

Studies of the nutrition of algae, both freshwater and marine, have been going on since the 18th century. However, progress was slow compared to other branches of biology, probably because of difficulties in culture and manipulation. To define the nutritional requirements of algae, they must be obtained in pure culture. As a consequence, progress in these studies had to await the development of improved laboratory equipment that would make this possible. As a result, during the 19th century algae were studied almost exclusively from the morphological and taxonomic points of view. By 1920 many species had been isolated in a bacteria-free state thus setting the stage for investigations under controlled conditions. Today the nutritional requirements of many species of algae are well documented.

Through the first half of the century limnology was more or less centered in the midwestern universities. Since World War II, however, teaching and reseach programs in limnology, with related centers of excellence in eutrophication, have been established in all the major geographic areas of the country. As a result, eutrophication problems peculiar to various regions can now be considered much more thoroughly than ever before. We hope these scientific resources will facilitate in many ways the control of this problem through sound management decisions. One such way is the continued synthesis of available information that bears on eutrophication and control possibilities.

An excellent beginning at such a synthesis occurred at the International Symposium on Eutrophication sponsored by the National Academy of Sciences and held on the campus of the University of Wisconsin in 1967. Proceedings of this symposium were published under the title "Eutrophication: Causes, Consequences, Correctives" (National Academy of Sciences, 1969). This went far to bring together much of the existing knowledge of eutrophication processes and controls. A second review document, "Eutrophication--A Review," was published in 1967 (Stewart and Rohlich, 1967). Together, these two publications emphasized the state of the art, pointing the way for future work. Their appearance at this critical time marks the year 1967 as an especially significant milestone in eutrophication and lake-restoration research.

THE LAST EIGHT YEARS

The period from 1850 to 1967 provides the historical preclude to the modern reaction to the eutrophication problem in the United State. No doubt, the manpower and dollars spent in the past 8 years to understand and cope with eutrophication far exceeds those spent in all of the preceding 117 years. The outlook today is that expenditure to activate remedial technology soon will be greater than dollars spent to develop new techniques.

Two factors lead to this conclusion: (a) several remedial approaches are available, and (b) the number of lakes to be addressed is very great. In the United States, excluding Alaska, there are about 100,000 lakes. At best, this is only an estimate because no standard definition of a lake is used by all states[1]. It is estimated, also, that 12,000-15,000 lakes are over 4.5 ha and that 10-20% are eutrophic. Generally, lakes in or near urban development are eutrophic, whereas many of those in relatively sparsely populated areas are more likely to be oligotrophic.

Today, one can still ask: What causes eutrophication? Many interacting factors contribute to the overall process. Productivity depends on solar radiation, temperature, lake-basin morphology, water-retention time, and perhaps most important, the availability of adequate nutrients. It is generally agreed that algae and higher aquatic plants require 25-30 different nutrients for growth. Large amounts of carbon, nitrogen, hydrogen, and phosphorus and smaller amounts of approximately 25 others, such as magnesium, calcium, boron, zinc, copper, molybdenum, and manganese, are necessary. In addition, vitamins such as B_{12}, thiamine, and biotin, and hormones play a part in nutrition. In theory, since all of the above are essentially for growth, the unavailability of any one could control eutrophication. Generally, however, nitrogen and phosphorus emerge as the critical elements in controlling aquatic plant nuisances.

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[1] Some states report, as lakes, bodies of water over 1.21 ha (3 acres); others, over 4.05 ha (10 acres); and others over 40.5 ha (100 acres).

The Carbon Problem

In the early 1970's a major controversy developed in regard to the relative importance of carbon, phosphorus, and nitrogen in regulating eutrophication. This controversy centered on a contention that carbon rather than phosphorus or nitrogen limits algal productivity in many aquatic ecosystems. Since phosphorus in detergents is linked to cultural eutrophication of lakes and streams, the controversy became emotionally charged following proposals to remove phosphorus from detergent formulations. Because of this, the American Society of Limnology and Oceanography sponsored a special symposium entitled "Nutrients and Eutrophication: The Limiting-Nutrient Controversy" (Likens, 1972). This symposium was held in February 1971 in an effort to provide a clear statement on the relative importance of various regulating or limiting nutrients in the eutrophication of aquatic ecosystems. The papers and discussion focused not only on phosphorus, nitrogen, and carbon, but also considered other nutrients and environmental factors that affect eutrophication. As various ideas, views, and data were openly and authoritatively debated, there emerged a general agreement that phosphorus is the critical limiting nutrient in most North American lakes and hence should be the center of focus for management programs. This expression in the published proceedings provides guidelines to the public and to officials concerned with lake protection.

Nutrient Loading

Preventive and remedial programs based on nutrient-control measures can be initiated effectively only after the origins of the nutrient supplies have been determined. To obtain an accurate nutrient budget, all sources must be considered, including all tributaries, industrial discharges, municipal discharges such as sewage and storm waters, precipitation, ground water, unchanneled surface runoff, and last but not least, feedback from the lake sediments. Synthesis of such a budget for even one nutrient is time-consuming, difficult, and expensive. Few accurate nitrogen and phosphorus budgets exist for U.S. lakes. Of these, the phosphorus budgets are generally more accurate than those for nitrogen.

Several actions are underway to improve our understanding of the relationship between lake loading and lake response. One program is the OECD[2] North American Project. Approximately 40 scientists from the United States and Canada are collecting and analyzing limnological data from selected lakes. Correlations of nutrient loading with mean depths and water-residence times are being examined to determine relationships of these factors to the prevailing trophic level in the studied lakes. The results of this study will be available soon.

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[2] Organization for Economic Cooperation and Development.

The U.S. Environmental Protection Agency (EPA) initiated "The National Lake Survey" in the summer of 1972. This project determines the location, severity, and extent of eutrophication in lakes and impoundments that act as receiving waters for municipal waste-treatment-plant effluents. At a cost of $7-8 million and a time span of at least 4 years, the study will examine 812 major lakes and impoundments in 48 states. For each lake the trophic state is estimated, and the sources and magnitudes of nitrogen and phosphorus supplies are identified, to judge whether reduction in phosphorus loading will restore or protect the lake.

To sample the lakes and measure limnological characteristics, EPA uses three helicopters equipped with special remote and contact sensors. Each lake is sampled, at multiple sites, three times during the growing season. The pontoon-equipped helicopters land on the lakes where probes are lowered into the water to measure dissolved oxygen, conductivity, temperature, and turbidity at different depths. Samples from various depths are analyzed for approximately 15 parameters. Algae are identified and counted. Algal assays are conducted on the lake waters to determine the productivity potential of the water and to assist in the identification of the limiting nutrient. The major streams entering or leaving the lakes are sampled for nitrogen and phosphorus. Stream flows are measured, and sewage treatment plant effluents are also sampled.

Another phase of the survey's work is to develop techniques to determine relationships between land-use patterns and nitrogen and phosphorus supply by using aerial photography. Land-use types will be correlated with the trophic condition of lakes and refined nutrient flux factors developed for various land-use types and geographic areas. This and other information from the survey has made it possible to focus on the following aspects:

1) Relationships between drainage-area characteristics and non-point source nutrients in streams (Figures 1 and 2);

2) quantitites of nitrogen and phosphorus in wastewater effluents;

3) the relationships of phosphorus and nitrogen to the trophic state of northeast and north-central lakes and reservoirs (Figures 3 and 4); and

4) an approach to a relative trophic index system for classifying lakes and reservoirs.

The final step of the survey will be the interpretation of the data for each lake and, in cooperation with appropriate state agencies, the derivation of subsequent recommendations for remedial action.

Algal Assays

Algal assays have been for some time an extremely valuable tool for evaluating water quality in relation to eutrophication. Many investigators

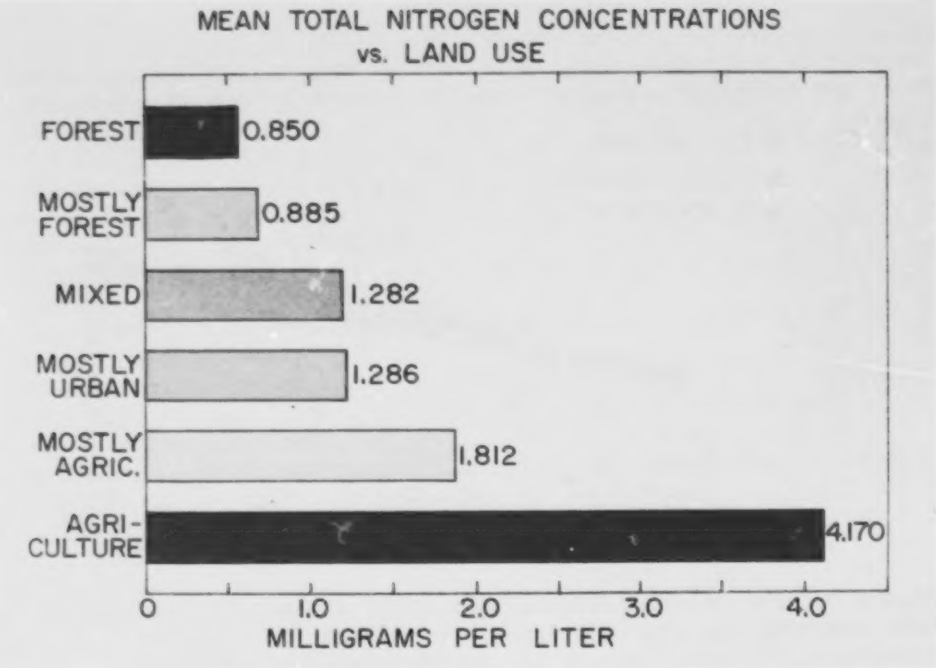

Figure 1. The relationship between mean total nitrogen concentrations in streams and land use in the Eastern United States.

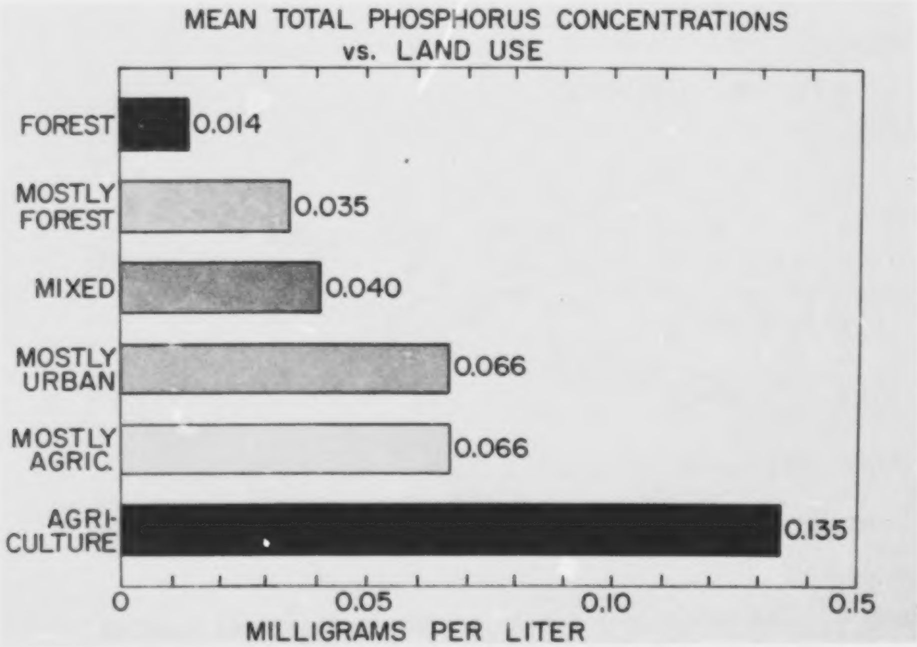

Figure 2. The relationship between mean total phosphorus concentrations in streams and land use in the Eastern United States.

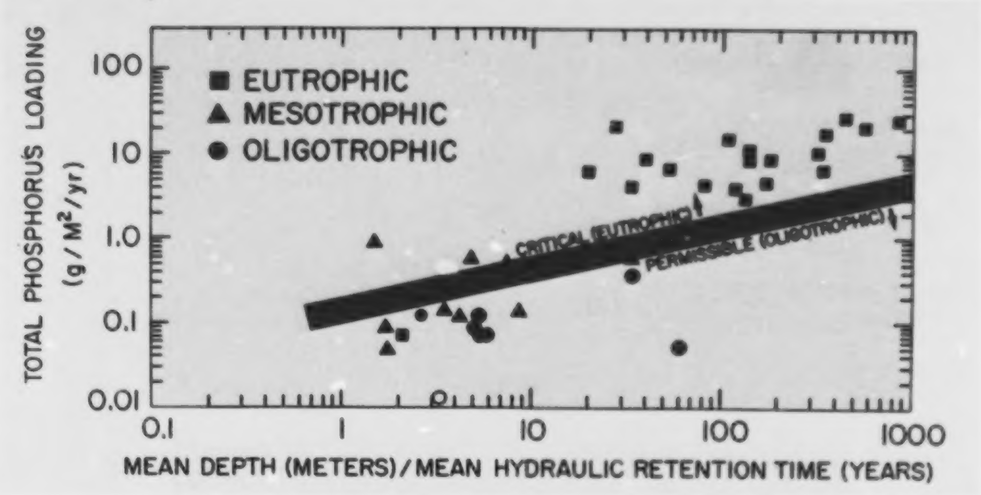

Figure 3. The relationship between total phosphorus loading, lake morphometry, and lake trophic condition for selected phosphorus-limited lakes in the Northeastern United States.

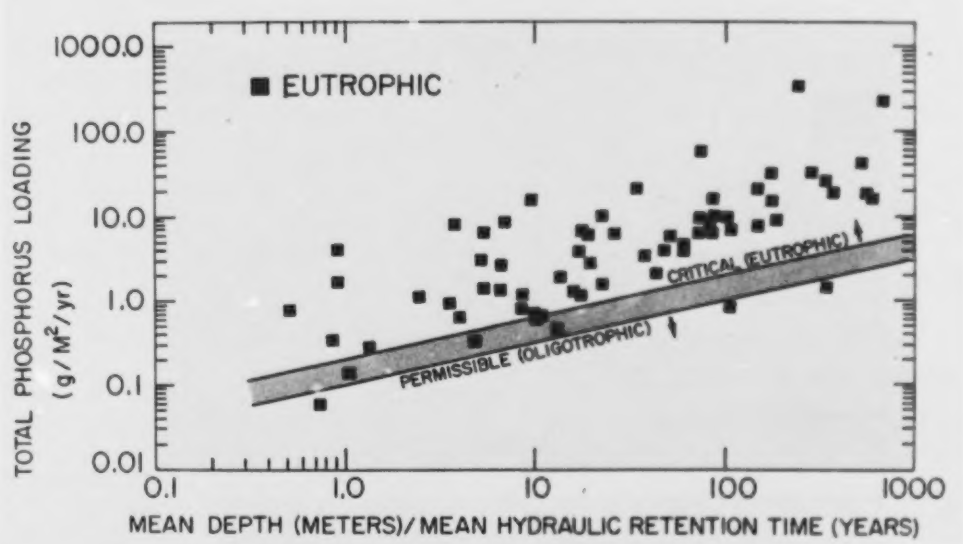

Figure 4. The relationship between total phosphorus loading, lake morphometry, and lake trophic condition for selected nitrogen-limited lakes in the Northeastern United States.

improvised algal assays to meet their specific needs, but because they were nonstandard, they offered no basis for comparing results among laboratories or among samples obtained from different geographic areas. Early in 1968 EPA prepared a tentative procedure for a proposed standardized algal growth test. It was intended to (1) identify and determine the availability of algal growth-limiting nutrients; (2) quantify biological response to changes in concentrations of algal growth-limiting nutrients; and (3) develop a rational framework for application of assay results to practical problems.

Early in the developmental effort it became apparent that emphasis should be placed on a static bottle-type test. In August 1971 the "Algal Assay Procedure: Bottle Test (AAP)" (National Eutrophication Research Program, 1971) was published after interlaboratory precision tests at eight laboratories showed excellent agreement in the data. It was then concluded that the bottle test had undergone sufficient evaluation and refinement to be considered reliable. It has been applied to numerous situations to assist in solving and understanding eutrophication problems. The assay procedure is included in the 14th edition of "Standard Methods for the Examination of Water and Wastewater" by the American Public Health Association, et. al.

Eutrophication Control

Since the early use of copper sulfate to control algae (Moore and Kellerman, 1904), an array of ecologically based procedures has evolved. What are today's options for controlling eutrophication? How can lakes be protected from further degradation and, equally important, how can eutrophic lakes be restored? Without question, the most promising preventive and restorative measure is to curb nutrient supply. This objective can be approached in several ways.

Nutrient Diversion--

A commonly used method to reduce nutrient input is to divert point-source nutrient-rich wastewater around or away from the receiving body of water. The early programs at Madison, Wisconsin, and Seattle, Washington, are well known. The same approach has been used more recently at Lake Sammamish, Washington, and Twin Lakes, Ohio. Although these lakes have improved, they have not responded to the same degree as Lake Washington.

As an alternative to diverting wastewater the effluents can be sprayed on the land and still protect the lake. This approach required much more land area than conventional treatment facilities; hence, when land is costly or not available, it may not be a viable remedy. Land requirement is about 1 ha per 300-400 population served. The process requires very little construction facilities, has a low energy requirement for operation, and can be easily automated for unattended operation at small facilities. Success is also very dependent on climatic factors, and year-round use will obviously be impractical in regions with severe winters.

The State of Michigan has completed a program of soils testing for phosphate absorption capacity, and New York State has undertaken such a program.

Processing organic wastes and returning them ultimately to the land is one of the most far-sighted methods for dealing with them. One of the largest demonstrations of this technique is currently underway at Muskegon, Michigan.

Another way to reduce the nutrient input is to remove specific nutrients by advanced waste treatment. Either nitrogen or phosphorus or both can be removed. One of EPA's current major research undertakings is a unique project to demonstrate the feasibility and dynamics of restoring a deteriorating lake by removing phoshorus from municipal wastewater flowing into it. Shagawa Lake, in northeastern Minnesota, was selected to demonstrate this technique. High inputs of phosphorus supplied by the lake shore city of Ely have caused excessive productivity and undesirable conditions in the lake. Ely, producing at a maximum about 3500 m^3 (1,000,000 gal) of wastewater daily, has had a municipal sewer system since 1901 and a secondary treatment plant since 1954. The effluent has always been discharged into Shagawa Lake. As a result, over the last 70 years, the lake has become increasingly eutrophic, a condition in great contrast to the near-pristine surrounding lakes. A $2.3 million tertiary waste-treatment facility, designed to remove more than 99% of the phosphorus in the wastewater from the secondary sewage treatment plant, was constructed with 95% financing by EPA. Full-scale operation began in early 1973. Phosphorus is removed by chemical treatment, primarily with lime and lesser amounts of ferric chloride, settling, and filtration. Only about 68 kg of phosphorus now enter the lake each year from this source, instead of the 6,800 kg before tertiary treatment. This plant is unique in the United States in removing phosphorus from all of the municipal wastewater to a residual of 0.05 mg/liter.

According to existing mathematical models of Shagana Lake, recovery should be rapid, very likely reaching a new phosphorus equilibrium in 1½ to 2½ years. However, when taking into account the phosphorus contained in the bottom sediment, and its exchange with the overlying water, additional time must be allowed for depletion of this nutrient source. Nevertheless, significant reduction in the phosphorus level of lake water has been noted, and the chlorophyll a concentration has been reduced from pre-treatment levels.

Product Modification--

Still another way to limit nutrient loading to lakes is to modify nutrient-rich products to reduce their growth-promoting potential. The best example is phosphorus compounds in detergents. It has been estimated that on a per capita basis 0.96 kg of phosphorus per capita year was utilized in the household and 0.24 kg per capita year was utilized in industry (Porcella et al., 1974).

On the average, roughly half of the phosphates entering U.S. streams come from municipal wastes and urban runoff. The other half comes from natural runoff, industrial and agricultural wastes, and animal feed lots. About half of the phosphates in domestic wastes are of detergent origin (Hatling and Carcich, 1973). Thus, detergents account for about one-quarter of the phosphates discharged into lakes and streams.

Onondaga Lake, New York, portrays the result of modifying detergent compounds. Following the implementation of local and state legislation in 1971-72 that limits the phosphorus allowed in detergents to 8.7% as P, a decrease of 54% in the concentration of total inorganic phosphate occurred in the Syracuse sewage treatment plant discharge to Onondaga Lake. The average total inorganic phosphate concentration in the lake also decreased by 57%. In the first full growth season after implementation of the law, the blue-green alga Aphamizomenon was newly absent in the succession of phytoplankton.

In-Lake Treatment--

Once the nutrients have entered a lake, the problem of eutrophication control is more complex. However, various control methods are under investigation. One can increase the nutrient output, immobilize the nutrients, withdraw nutrient-rich hypolimnetic waters, or dredge to remove nutrient-rich sediments. One may also treat the symptoms, such as nuisance algae, plants, and fish, by applying poisons or toxins, by harvesting, or by biological grazing.

Algicides and herbicides--Chemical treatment has been a widely used method to improve the appearance and usefulness of lakes. It is intended to limit specific populations of organisms, such as blue-green algae, higher aquatic plants, or unwanted fish populations that become nuisances. The chemicals vary in their cost, effectiveness, toxicity, and persistence. In any event, the result is only temporary or "cosmetic" in that it treats only the symptoms and not the cause of the problem. In addition, decomposition of the target species serves to regenerate nutrients that allow for continued biological development of the same or different populations.

Mechanical harvesting--A method of eutrophication control that is being used with decreasing frequency is the harvesting of plants or animals from the lake. The product may be stumps or sunken logs as at Marion Pond, Wisconsin, rough fish, or higher aquatic plants. Weed-harvesting equipment is available, but attempts to develop equipment and procedures to harvest algae have not been successful. Harvesting obviously removes some nutrients from a body of water, but the amount of phosphorus and nitrogen removed is exceedingly small. In the makeup of plants an average reported value for P is 0.24% and for N is 2.3%, dry weight concentration. A recently published report (Peterson, Smith and Malueg, 1974) on a harvesting study on Lake Sallie, Minnesota, states: "Perhaps the most significant conclusion to be derived from this study is that continuous harvest of aquatic plants from Lake Sallie during the growing season could not offset the high loading of phosphorus and nitrogen. The net-weight harvest of 428,000 kg of plants was successful in removing only 1.3% of the total phosphorus to the lake, or 1.03% of the phosphorus contained in the water volume of the lake during the fall circulation period.

In spite of this, harvesting often can be justified on the basis of aesthetic values alone. Research by the University of Wisconsin on Lake Mendota indicates that one harvesting will reduce the amount or regrowth to about 50% of the controls, two harvests will result in about 75% reduction, and three harvests almost totally eliminate the plants for that year. The researchers recommended two harvests, one in June and the other in July, for that climate. None of the treatments had an appreciable effect on the subsequent year's growth. So far little is known of the effects of harvesting of higher aquatic plants on the phytoplankton.

A demonstration in the State of Florida provides another example of weed harvesting. The St. John's River is the largest river entirely within the state, approximately 480 km long. Problem weeds such as water hyacinths occur in areas where water use for navigation is extensive. The weeds also retard irrigation and drainage and reduce game fish and water fowl. The decomposition of detritus from these plants also depletes the oxygen from the lower waters. Control of water hyacinths on the St. John's River has been carried out with chemicals for a long time. Now researchers are experimenting with harvesting techniques. In Florida alone, more than 40,000 ha of water are covered with water hyacinths, despite extensive and continuous programs of control by various governmental agencies.

Dredging--A procedure that can be thought of as an extension or modification of harvesting is dredging of the sediments of a eutrophic lake. Perhaps in many lakes the sediments are an important source of nutrients that may be cycled to the overlying waters, especially at certain times of the year. In theory, dredging would remove this nutrient source, but there are several problems, not the least of which is disposal of dredged material. These problems are being addressed in an extensive research program recently undertaken by the U.S. Army Corps of Engineers.

Dilution or Flushing--Another method of eutrophication control is flushing or dilution. Use of this method is limited by the availability of fresh water. Two nutrient dilution procedures have been attempted: (1) pumping water out of the lake, thus permitting increased inflow of nutrient-poor groundwater, and (2) routing additional quantities of nutrient-poor surface waters into the lake. The first has been used at Snake Lake, Wisconsin. The second has been tried in several places. One of the most successful experiments was done at Green Lake, Washington, where, after 5 years of flushing (plus some initial dredging), the blue-green algal standing crop was suppressed, with elimination of Aphanizomenon. Flushing has also been tried on a small scale at Moses Lake, Washington.

Aeration--It is also possible to immobilize nutrients in eutrophic lakes through aeration of hypolimnetic waters where large reservoirs of phosphorus may accumulate. Aeration methods generally fall into two groups: those that destratify the lake and thus affect all depths, and those that aerate only the bottom waters and do not destratify the lake, i.e., hypolimnetic aeration. When destratification is accomplished, the lake becomes isothermal with oxygen present at all depths and other chemical conditions fairly uniform. Hypolimnetic aeration has certain advantages over destratification. Nutrients are not upwelled into the surface waters where they may promote algal growth. Further, hypolimnetic aeration permits the establishment of a cold-water fishery such as trout or salmon, whereas destratification may preclude such a fishery by eliminating the cold-water region.

Examples of aeration projects for eutrophication control are at Cline's Pond, Oregon, where destratification aeration was used; at Lake Waccabuc, New York, where the "Limno" hypolimnetic aerator is being used; and at Ottoville Quarry, Ohio, where hypolimnetic aeration is achieved by a process called "side stream pumping".

Nutrient inactivation--A promising approach for a wide range of situations is nutrient inactivation. This involves treatment of lake waters in situ with a chemical to precipitate phosphorus. Inactivant materials that have shown particular promise in laboratory and field studies are aluminum, zirconium, and fly ash. Experiments with aluminum compounds are presently being conducted on lakes in Wisconsin, New England, Ohio, and Washington. Zirconium is being tested in a controlled pilot field study in Oregon, and a similar experiment, utilizing fly ash, is under way in Indiana. It is anticipated that such treatments will be particularly efficacious in lakes with very long retention times.

Hypolimnetic withdrawal/selective discharge--Hypolimnetic withdrawal has been used to improve dissolved oxygen conditions near the bottom of a lake and to increase nutrient export. In bodies of water that stratify, this technique permits the removal of anaerobic, nutrient-rich deep waters. The technique is suitable for waters with outlet controls, such as reservoirs, or in lakes with surface withdrawals by installation of a siphon from a point of maximum depth. The surface discharge is, or can be, completely blocked off. This technique has been used in Wisconsin, Ohio, and other states. A potential problem with its use is the triggering of increased macrophytic growth and low dissolved oxygen in the downstream channels.

Drawdown--Sediment exposure and desiccation via lake drawdown has been undertaken on impoundments for various purposes. In favorable sites this procedure can reduce the rooted aquatic plants by desiccation. The effect of drying on sediment chemistry and possible nutrient release is now being studied, particularly in Florida and Louisiana. Presently 13 Louisiana impoundments are being managed by water drawdown to aid in control of aquatic vegetation and fish populations.

Biological control--The control of particular problem species by manipulation of biotic interactions has been a much desired goal in recent years. Evaluation of biological controls has been limited, however; most testing has been done in the laboratory or experimental ponds. Considerable publicity has been given to programs which have sought to decrease the density of weed species through the introduction of host-specific predators, and a great amount of research has been expended in predator control of macrophytes. One such program has been relatively successful: the flea beetle has reduced populations of alligator weed considerably in some areas of the Southeast.

A somewhat less specific herbivore, the grass carp or white amur, has been released in numerous lakes in Arkansas, where it has apparently been able to successfully control undesirable submerged weeds. Its widespread introduction into this country, however, is still the subject of much apprehension and study.

Similar control programs involving crayfish, a specific weevil for water hyacinth, and other insects are currently under investigation. Aquatic mammals such as the manatee and other animals such as snails and swans have also been tried. Although most of these animals have been somewhat effective on a local basis, few are effective over a broad geographic range. The need to carefully consider and anticipate the total effects of introduced or exotic species on the natural ecology is well known.

Relatively little work has been done with biological control of algal populations. Bacteria and viruses have been isolated that destroy blue-green algae, but to date only laboratory tests have been conducted. No full-scale, in-lake treatment has ever been tried in the United States. The control of undesirable macrophytes with plant pathogens, mostly fungi, may show some potential and is currently being evaluated on a small scale.

Biomanipulation, or facilitating desirable interactions among different segments of the whole ecosystem, has long been a desirable goal. Some possibilities in this direction are under study. Attempts are being made to reduce phytoplankton abundance by increasing the number of grazers by either direct innoculation or by controlling the zooplankton by disease or carnivorous fish introductions. Attempts to exploit the competitive or inhibitive reactions among aquatic weed species are also being studied as possible control measures.

Legislation

Lake-restoration measures are in a very early stage of development. Much of the technology is still being applied in laboratories, in experimental ponds, or in pilot lake studies. No technique can be applied indiscriminately to every problem lake; each must be studied and evaluated sufficiently to assure that the most appropriate course of action is taken. Obviously, a whole range of remedial methods must be made available. Public Lake 92-500, the Federal Water Pollution Control Act Amendments of 1972, will certainly help in this regard because it authorizes funds to support state programs for lake restoration. The Congress has appropriated $4 million to EPA for this purpose; approximately 10% of the funds have been designated for evaluation purposes. It is expected that funding will be increased next year.

To limit fertility in lakes, several states have passed laws setting forth regulations affecting nutrient loading. Some of them are the following:

Minnesota has passed legislation to set effluent standards for phosphorus in municipal discharges. If the discharge enters a lake directly, the phosphorus concentration must not exceed 1.0 mg/liter; if to a lake via a river, 2.0 mg/liter. Similarly, Illinois has adopted an effluent standard of 1.0 mg/liter phosphorus for discharge to Lake Michigan.

In Iowa, laws were passed in 1971 that provide for mandatory soil conservation. Iowa's Conservancy District Act established conservancy districts and declared soil erosion resulting in siltation damage to be nuisance. The act also directed the commissioners to establish soil-loss limits for their districts.

The Wisconsin Shoreland Protection Statute authorizes and requires counties to adopt pollution-control regulations for the shoreland areas. The law sets out zoning, sanitary code provisions, and subdivision regulations.

Indiana, Iowa, and Minnesota have adopted various kinds of farm-animal-waste regulations, aimed chiefly at problems related to large feedlot installations.

A number of states have acted to limit the phosphorus content of detergents. New York and Indiana passed such laws in 1971. The New York law reduced the phosphorus content to 8.7% by January 1972 and to a trace by July 1, 1973. The Indiana law limited phosphorus to approximately 5% after the beginning of 1972, and to 3% after January 1973. The law has changed in 1972 to read 8.7% after January 1, 1972 and to zero after January 1, 1973, Indiana thus becoming the first state to completely ban phosphorus in household laundry detergents.

Laws limiting phosphorus in detergents were also passed in Florida, Maine, Michigan, Minnesota, Connecticut, and Oregon, as well as in Chicago, Illinois, Akron, Ohio, and Dade Country, Florida.

The Environmental Protection Agency is presently drafting phosphorus criteria for recreational waters. Although the agency does not propose a limit of acceptability for phosphorus, it gives guidelines for the establishment of total phosphorus criteria in receiving waters. These will include both a concentration, which prescribes maximum acceptable levels, and a loading value in the form of an annual allowable specific loading to the receiving water.

The United States and Canada joined together on April 15, 1972, under the Great Lakes Water Quality Agreement "to restore and enhance water quality in the Great Lakes system" (Great Lakes Water Quality, 1972). Annex 2 of the agreement pertains to control of phosphorus. It specifies effluent requirements for municipal waste treatment plants, goals for industries, and reductions in input from animal husbandry operations.

THE FUTURE

The science of limnology and the study of eutrophication have come of age since 1900, but considerable work still remains if this nation is to have clean lakes and streams. The following statements identify specific areas where intensified research is needed if we are to succeed in these goals:

1) Develop and utilize remote sensing so that water bodies can be quickly trophic level.

2) Develop methods to examine and manage lakes as part of an entire watershed.

3) Delineate the role of sediments as a source or sink of nutrients, to facilitate predictions of impact on lake recovery prior to initiating control or restorative practices.

4) Evaluate the role of the thermocline as a barrier to the transfer of chemical and biological material and possibilities for beneficial manipulation.

5) Understand the reasons for seasonal succession of algal types and, in particular, the reasons for the appearance and dominance of blue-green algae.

6) Determine interactions between macrophyte and phytoplankton populations and the effects on one when the other is manipulated.

7) Develop methods to control macrophytes to achieve a balance with desirable uses of the lake.

8) Develop and evaluate methods of aquatic ecosystem mangement through biological manipulations so that the water body produces the most desirable product.

9) Evaluate useful products derived from harvestable material from water bodies. Such products would include soil conditioners, pharmaceutical materials, animal feed, and energy source.

10) Develop techniques to predict the success of control or management methods on lakes through mathematical modeling.

11) Determine the socioeconomic aspects of cultural eutrophication and lake restoration including recreational impact, effects on commercial fisheries, public health, and cost of water treatment.

Despite the research and refinements that are still to come, many significant advances in eutrophication and lake-restoration research have been made. Some techniques now available can be and are being used with reasonable success on individual lakes. Other options need to be developed. The continuing interdisciplinary interest in this area of water-resources mangement is heartening. Scientists, engineers, economists, and others working together will ultimately find solutions to save and protect our aquatic habitats. We must act to protect mankind's great natural heritage without destroying it. In trying to find the ways to do it we must cherish the words of Chief Seattle:

> "The earth does not belong to man; man belongs to the earth."

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SECTION 10

DETERMINING THRESHOLD AND BIOLOGICALLY DANGEROUS CONCENTRATIONS OF BLUE-GREEN ALGAE IN WATER BODIES

L.A. Sirenko, A.Ya. Malyarevskaya, and T.I. Birger[1]

The economic activities of man have caused eutrophication in many water bodies. One of the significant results of this is a change in the species composition and number of aquatic organisms in an affected area. This often causes a disturbance in the regulation processes in the ecosystem of the water body. The blue-green algae bloom may be the most important example of disturbance in the ecological balance under the influence of anthropogenic factors. In this case, excessive development of individual species of algal flora determines the whole complex of the internal water body processes and the ecosystem's final biological productivity. As is known, in their vital processes algae excrete into the environment about 30% of the total carbon absorbed by them during 24 hours or about 40% of the daily pure photosynthesis production.

Excreted products include: Organic acids which determine the environmental buffer action and its pH; amino acids and peptides, which contribute to the formation of the complex and lower the toxicity of heavy metals; polysaccharide substances which adsorb on their surface the most varied types of ions, aldehydes, terpenes, polyphenol compounds; and also other biologically active substances which dominate biological activity.

Toxins found in individual species of algae (blue-green, flagellateae, peridium) also play a very important role among exogenous metabolites.

Blue-green algae excreting some substances during life processes, and decomposition not only form a definite biotic environmental background, but also change the hydrochemical indices of the environment if their accumulation is considerable. In this case, the quantity of oxygen diminishes, the content of carbonic acid increases and the environmental reaction changes. In other words, the effect of algae on the aquatic organisms depends on a whole complex of biotic and abiotic factors.

In addition to this direct influence, blue-green algae may also affect the aquatic organisms indirectly. Namely, the substances they excrete may intensify or weaken to a considerable degree the action of different chemical substances entering the water body. This effect may result in the formation of complex compounds, suppression or stimulation of the bacterioflora, and the appearance of free radical compounds if photosynthetic oxygen and some other factors are present in the environment.

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[1] Institute of Hydrobiology, Academy of Sciences, Ukrainian SSR.

This indicates that when determining the Maximum Permissible Concentration for toxic substances, it is necessary to take into account the influence of exogenous metabolites of algae on toxic compounds and likewise it is necessary to determine biologically dangerous concentrations of the natural metabolites for fish and other aquatic organisms.

This information examines the possibility of determining, for fish, the threshold and biologically dangerous concentrations of metabolites of blue-green algae that cause "blooms".

During cell reproduction of Microcystis, the main stimulus for algae "blooms", the environment accumulates exogenous metabolites which have a high biological activity (Goryunova, 1966; Sirenko, 1971). They include polyphenol compounds, and likewise polynucleotides. We determine that in concentrations up to about 0.28 ppm, phenol compounds suppress the growth of other examples of algal flora, not affecting the life processes of the species-producer. When the concentration of phenol compounds increases to 1.3 ppm, autoinhibition of the algal growth and reproduction is observed.

This indicates the role of the metabolite concentrations in the cell-division regulation processes; in this case, it concerns the algae cells in culture and natural conditions. If we changed the concentration, we would be able to regulate to a definite limit the number of algae cells in a population.

The metabolites produced by algae are not of less importance for the life processes of other aquatic organisms entering into communities and important in the self-purification processes. For example, the effect of blue-green algae metabolites on the decrease in the producing capacity of Daphnia was shown (Braginskiy et al., 1965).

As for fish, there has been little study of the influence of natural blue-green algae metabolites on them. The threshold and biologically dangerous concentrations of these metabolites for all practical purposes, have not been formulated.

At present, when determining the maximum permissible concentration of some basically artificial substances entering the water body, the following criteria are usually considered (Stroganov, 1972):

1. Mineralization processes of organic substances.

2. Oganoleptic indices of water and water organisms (especially of fish).

3. Survival, growth, reproduction, fertility, and quality of the aquatic organisms' progeny.

All the enumerated criteria most completely characterize the ecological and toxicological situation in the water body and are widely used to determine the maximum permissible concentration of artificial substances.

In view of the necessity to develop express methods which would help to detect the aquatic organism-metabolism links changing in the first instance under the influence of toxicants, a study of the biochemical mechanisms of toxicant effect on the aquatic organisms has been planned. There are some data affirming that the effect of many toxicants is the result of effects on enzyme processes in the aquatic organism. For example, an anticholinesterase effect of phosphorus organic compounds on fish has been ascertained (Metelev, et al., 1971).

The investigation carried out in our Institute (Malyarevskaya, Birger, Arsan, Solomatina, 1973) give an idea of the threshold and biologically dangerous concentrations of biological toxicants for fish (e.g., effect of blue-green algae _Microcystic_ _aeruginosa_ Kutz. emend. Elenk). The experiments were carried out from 1964 to 1974 in laboratories and in water bodies exposed to a heavy "bloom" caused by mass development of blue-green algae. Effects of various blue-green algae concentrations on fish (pike perch _Lucioperca_ _lucioperca_ L., perch _Perca_ _fluviatilis_ L., ide _Leuciscus_ _idus_ L., crucian _Carassius_ _carassius_ L., and _Hypophthalmichtys_ _molitrix_ _Val._) were studied.

In long-term experiments, small quantities of algae were applied (0.03-0.30 g/liter). Since the fish did not die, they were conditionally named "nonlethal". When more considerable concentrations (0.6 - 5.0 g/liter) were used in acute experiments, the fish died within a period of 6-64 hours, depending on the fish species and algae concentration and condition (living, decaying). Such algae concentrations were called "lethal".

The biochemical composition of fish (content in their bodies of dry organic substances, ashes, protein and its amino acid composition, lipids, vitamins B_1, B_2 and enzyme activity of thiaminase, cholinesterase, transaminse and content of nicotinamide coenzymes) and also the fish metabolism (interchange of gases and nitrogen exchange) have been investigated and the effect of various algae concentrations on them has been ascertained.

The effect of blue-green algae on fish is conditioned by the complex of biotic and abiotic factors which includes the effects of metabolism products, algae decomposition, and changes in the hydrochemical indices in the environment.

Non-lethal algae concentrations (0.03-0.30 g/liter) do not cause the death of fish, but if a fish inhabits waters characterized by such a quantity of algae (especially 0.3 g/liter), it results in determinable changes in the metabolism, i.e., suppression of plastic processes and intensification of energetic processes. The growth of dry and organic substances and protein in fish drops and nitrogen consumption is reduced. The expenditure of nitrogen for energetic processes increases (Table 1).

Considering that the most evident negative deviations from the control are observed in fish in concentrations of 0.30 g/liter, this quantity of algae may be considered a threshold concentration.

Lethal concentrations of blue-green algae caused more significant changes in the biochemical composition and the fish metabolism. The data show that fish lost dry and organic substances, protein and lipids. The nitrogen balance was negative. Respiration intensified at a stage of heightened movement activity and slowed down before death. A change in the content of free amino acids (Table 2) and protein hydrolysates amino acids, and also transaminase activity, pointed to the protein synthesis disturbance. A decrease in nicotinamide coenzyme oxidized forms confirmed that considerable changes in oxidation-reduction processes in tissues took place.

Given the effect of the blue-green algae lethal quantities on fish, changes in the thiaminase enzyme activity and total thiamine content (vitamin B_1) were most significant. A thiaminase activity increase of 21-40% in organs and tissues of fish influenced by the blue-green algae and thiamine content drop of 38-50% in comparison with the control value, resulted in a convulsion stage. A thiaminase activity increase of 28-55% and a thiamine content drop of 49-74% caused the death of fish (Table 3). A number of experiments, in which thiamine chloride injected at the initial paralysis stage stopped the convulsions and prolonged the fishes' life, corroborate that avitaminosis B , given the influence of lethal concentrations of blue-green algae, is the reason for the fishes' death. This is also indirectly confirmed by the fact that thiaminase activity in fish in natural conditions (ponds, reservoirs) during the "bloom" period was heightened, but the total thiamine content was lower than in autumn when no "blooms" were observed (Table 4).

A shortage of vitamin B_1 in organs of fish, given the influence of lethal concentrations of blue-green algae, causes a disturbance in all metabolism processes in which it participates. As mentioned above, in this case the protein exchange, and likewise biosynthesis of lipid structure compounds and normal transformation of substances in Krebs' cycle, are disturbed. Changes in biochemical processes result in disturbances of some functions. In particular, B_1-avitaminosis involves changes in the nervous system's functional state. The latter is corroborated by a drop in the cholinesterase activity in the fish's brain, given the influence of lethal concentrations of blue-green algae. Some other symptoms typical of the thiamine shortage are also observed in these fish, namely: Disturbance in liver functions, the alimentary canal, and cardiovascular system; hemorrhage in organs; and pathological changes in blood-formation. Blood analyses of fish caught in waters covered with "bloom" areas support the latter (Komarovskiy, 1970).

Thus specific phenomena, e.g., B_1-avitaminosis, resulting in a number of nonspecific changes--in particular, non-coordination of energetic and

TABLE 1. CHARACTERISTICS OF NITROGEN EXCHANGE IN THIS YEAR'S GROUP OF PERCH

Concentration of blue-green algae (g/l of live weight)	Physiological state of algae	Average daily nitrogen ration of 1 fish (mg)	Nitrogen accumulated per 24 hrs in organism of 1 fish (% of the average daily nitrogen ration)	Nitrogen excreted during 24 hrs by one fish (% of the average daily nitrogen ration)		
				with liquid excretions	with excrements	total excreted nitrogen
--	Control	2.8	30.0	46.6	23.4	70.0
0.03	Living	2.2	10.9	75.9	13.2	89.1
0.30	Living	1.9	12.3	71.8	15.9	87.7
0.30	Decomposed	1.4	2.8	84.6	12.6	97.2

TABLE 2. CONTENT OF FREE AMINO ACIDS IN ORGANS AND TISSUE OF IDE YEARLINGS (mg/g of Fresh Tissue)

Amino Acids	Indices	Intestine M ± m	Intestine diff	Liver M ± m	Liver diff	Muscles M ± m	Muscles diff
Cystine	Control	0.43±0.04		0.34±0.10		0.09±0.01	
	Experiment	4.74±0.13	31.3	1.29±0.04	8.46	0.26±0.04	4.12
Lysine	Control	0.17±0.02		0.29±0.05		0.93±0.07	
	Experiment	0.13±0.02	0.4	0.31±0.03	0.78	0.32±0.05	6.5
Histidine	Control	0.23±0.01		0.13±0.05		--	
	Experiment	0.31±0.01	2.6	0.25±0.00	2.29	--	--
Agrinine	Control	0.34±0.10		0.42±0.01		0.05±0.01	
	Experiment	0.17±0.03	1.6	0.31±0.07	1.26	0.04±0.08	5.5
Asparagine Acid+Serin	Control	0.68±0.09		0.22±0.03		0.33±0.01	
	Experiment	0.52±0.13	1.0	0.64±0.14	2.94	0.36±0.08	0.3
Glycine	Control	0.13±0.02		0.04±0.00		0.16±0.01	
	Experiment	0.58±0.06	7.38	0.11±0.01	4.93	0.12±0.02	0.23
Glutamine Acid+Treonine	Control	1.08±0.14		0.15±0.04		0.46±0.04	
	Experiment	1.43±0.23	1.15	2.01±0.15	11.99	0.13±0.01	6.22
Alanine	Control	0.55±0.03		0.09±0.02		0.50±0.06	
	Experiment	0.75±0.04	4.0	0.36±0.04	5.87	0.08±0.01	6.38
Tirosine	Control	0.08±0.01		0.08±0.00		0.10±0.02	
	Experiment	0.04±0.01	0.49	0.50±0.01	47.8	0.07±0.01	0.6
Metionine+ Valine	Control	0.49±0.01		0.20±0.00		0.03±0.02	
	Experiment	0.03±0.01	1.44	0.13±0.02	5.0	0.03±0.05	0.47
Phenilalanine	Control	0.16±0.01		0.05±0.00		0.04±0.00	
	Experiment	0.07±0.01	9.44	0.17±0.04	2.91	0.03±0.01	0.85
Leicine	Control	0.19±0.06		0.01±0.00		0.04±0.00	
	Experiment	0.17±0.03	0.36	0.12±0.03	9.9	0.06±0.01	1.82

TABLE 3. EFFECT OF LIVING BLUE-GREEN ALGAE (5 g/1) ON CONTENT OF TOTAL THIAMINE (μg/g) AND THIAMINASE ACTIVITY (μg/hr) IN LIVER AND INTESTINE OF FISH

Fish Species	Control		Initial state of paralysis		Death stage	
	Total Thiamine M ± m	Thiaminase Activity M ± m	Total Thiamine M ± m	Thiaminase Activity M ± m	Total Thiamine M ± m	Thiaminase Activity M ± m
			LIVER			
Pike perch	4.70±0.20	709.23± 8.50	1.89±0.10	992.21±16.00	1.21±0.10	1095.80± 8.75
Perch	7.27±0.23	565.64±25.00	5.53±0.26	775.04±17.00	3.45±0.18	841.14±22.70
Ide	4.62±0.17	663.22±21.80	2.65±0.15	808.23±16.40	1.60±0.16	861.17±22.30
			INTESTINE			
Pike perch	4.29±0.10	517.64± 9.54	1.97±0.10	720.62± 1.08	1.28±0.10	777.69± 1.80
Perch	3.32±0.18	699.14±18.00	1.90±0.10	849.41±28.60	1.67±0.28	901.83±39.30
Ide	4.71±0.23	530.34±26.00	2.22±0.13	661.42±22.00	1.42±0.13	767.33±24.40

TABLE 4. EFFECT OF BLUE-GREEN ALGAE ON CONTENT OF TOTAL THIAMINE (μg/g) THIAMINASE ACTIVITY (μg/hr) IN LIVER AND INTESTINE OF FISH

Indices / Fish Species	Autumn (no "bloom")		Summer ("bloom" period)	
	Total thiamine M±m	Thiaminasae Activity M±m	Total thiamine M±m	Thiaminasae Activity M±m
		LIVER		
SCARDINIUS erythrophtalnus L.	7.13±0.31	363.07±6.55	4.62±0.22	550.66±65.40
Roach	7.37±0.40	438.18±4.82	4.60±0.63	614.75±5.50
Bream	7.34±0.84	445.93±9.20	4.40±0.25	538.81±5.70
		INTESTINE		
SCARDINIUS erythrophtalnus L.	3.83±0.20	280.10±5.20	2.31±0.18	347.00±8.00
Roach	2.88±0.30	384.12±9.00	1.46±0.10	507.79±5.80
Bream	3.20±0.20	354.92±3.13	1.72±0.14	461.67±13.10

plastic processes and changes in the above mentioned factors including blood analysis--develop in the fish when exposed to lethal concentrations of blue-green algae.

A question arises as to symptoms (specific or nonspecific) that may serve as indicators when determining biologically dangerous concentrations? Oviously, specific changes, and, in the present case, changes in the thiaminase activity and the total thiamine content under the influence of blue-green algae, must serve as indicators. Judging from our experimental data, biologically dangerous concentrations of blue-green algae must range from 0.3 to 0.6 g/liter of raw substances.

However, it is important to remember that biologically dangerous concentrations of blue-green algae may change, depending on the effect of the algae's natural metabolites and synergism or antagonism with other biotic or abiotic water substances. In particular, the toxicity of blue-green algae will depend both on a series of chemical indices (temperature effect, content of oxygen in water, carbon dioxide, the presence of salts of such metals as manganese, zinc and lithium) and on the physiological state of algae cells (living, dead, decomposing). Thus, in our experiments, decomposing algae proved to be more toxic for fish.

The nature of an aquatic organism's reaction to the algae is important. Thus, predators are the first to react to the algal toxicant effect because they are organisms characterized by a more intensive metabolism and belong to the final link in the trophic chain. When estimating natural toxicants, it becomes necessary to consider indicator organisms.

We have data showing that anlysis of the biological toxin effect requires that we examine indicator organs which change more appreciably and begin to show changes at an earlier period of time. In experiments investigating the effect of lethal concentrations of blue-green algae on fish, the liver may be considered such an organ. The reversibility of fish intoxication is a very important problem for man and animals.

No doubt, several metabolic changes observed in threshold concentrations are reversible. Judging from our observations, even the changes in fish metabolism which occur in the fish under the influence of lethal concentrations of blue-green algae are reversible at early stages. Namely, respiration and several biochemical indices in fish transferred to pure water become normal. It is assumed that in the absence of sizable algal concentrations, metabolic processes in fish organisms will be normalized since algal concentrations may not only increase but also decrease due to the fact that the wind concentrates or disperses them in the water body. The important problem is the degree that normalization will affect the enzyme systems, and whether the thiaminase activity is lowered enough to avoid Gaff's disease if the fish is consumed by man or other animals. According to our data (Birger, Malyarevskaya, Arsan, 1972) the disease is an acute B_1-avitaminosis.

In addition, since each lethal concentration needs a definite period of time for action, short-term effects of even considerable algal concentrations do not always result in the fishes' death, but can change their metabolism and that will affect in the future the species reproduction.

A considerable effect of algal concentrations in the environment on the metabolism indices of some fish species is seen in an example of blue-green algae "bloom" stimuli. As a result of the investigations, threshold concentrations of algae accumulation in the environment have been determined. Exceeding those threshold concentrations appreciably results in negative effects of algae on vital activity of fish even in an environment completely deprived of other chemical pollutants.

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SECTION 11

TOXIC ORGANIC RESIDUES IN FISH

Howard E. Johnson

INTRODUCTION

The distribution of synthetic organic chemicals in the environment has emerged as a major problem of industrialized nations throughout the world. The discovery of widespread environmental contamination by DDT and dieldrin has led to the recognition of many other environmental contaminants including such industrial chemicals as polychlorinated biphenyls (PCB), pthalate esters, and hexachlorobenzene.

The development of sophisticated analytical techniques and intensified chemical monitoring efforts has shown that a wide variety of synthetic organic chemicals or their degradation products is present in the aquatic environment. As many as 40 potentially hazardous chemicals have been identified in some rivers that receive domestic and industrial effluents (Kleopfer and Fairless, 1972; Hites, 1973). Significant contamination may also be occurring in some regions because of chemical fallout from the atmosphere. The ecological and public health hazard of these contaminants is largely unknown, but the potential effect is considerable.

PRODUCTION AND DISTRIBUTION

The problem of environmental contamination is increased because of the magnitude of commercial chemical production. The United States has increased its production of chemicals by nearly 10% a year with present production exceeding 140 billion pounds. As many as 500 new chemicals are produced each year with little or no knowledge of the potential hazard of their behavior in the environment (Lee, 1964). Some compounds have highly toxic, carcinogenic, or mutagenic properties that may be especially damaging if they are accumulated in aquatic systems. In some instances the degradation products or metabolites may be of equal or greater consequences.

Aquatic ecosystems are especially vulnerable to the effects of chemical pollutants. Acutely toxic concentrations resulting from accidental spills or direct application have caused extensive fish kills over broad areas of the environment, but many chemicals occur in the environment at concentrations that are not directly lethal to fish. These compounds are distributed as microcontaminants, i.e., concentrations of a few parts per million or less, in various substrates within the aquatic ecosystem. Because of their very low concentration, such chemical contaminants may not be detected until they appear in undesirable levels within some trophic level. Serious ecological damage or effects on human health have sometimes occurred before we have taken action to prevent further contamination. An example is the environmental mercury problem, which first received worldwide attention when human beings were poisoned by eating contaminated fish and shellfish during the 1950's in Minamata, Japan.

EFFECTS ON FISHERY RESOURCES

Synthetic organic chemical residues that accumulate in aquatic organisms can have far-reaching effects on an entire fishery resource. In particular, the problem is exemplified by the impact of polychlorinated biphenyls (PCB) on fishery resources of the Great Lakes.

Occurrence and Accumulation

Polychlorinated biphenyls are a widely used class of chlorinated hydrocarbons found in a variety of manufactured products and in many industrial processes. Environmental monitoring has shown that PCB are distributed throughout the Great Lakes ecosystem, but the highest concentrations are generally found near industrial and urban area. Some specific industries are known to discharge PCB in their effluents, but non-specific sources such as municipal wastewater effluents are more difficult to control. Atmospheric contributions in the form of rain, snow, and particulate fall-out also may be significant.

Concentrations of PCB in the Great Lake waters are generally only a few parts per trillion (nanograms per liter), but because of biological concentration residues in some fish exceed 20 parts per million (milligrams per kilogram). Laboratory studies indicate that fish can accumulate PCB by more than 40,000 times the exposure concentration (Stalling and Mayer, 1972). The residues are most concentrated in the lipids of body tissue. Careful monitoring studies have shown that residue concentrations vary with different species in proportion to their fat content. The highest concentrations are found in mature salmon and trout just before or during their spawning migration (Veith, 1975).

It has been suggested but not proven that PCB and other chemical residues accumulated in the eggs are responsible for high mortalities of some fish during the early stages of development. Mortality of young salmon has been high where the eggs contained PCB, DDT, and some other chemical residues (Johnson and Pecor, 1969; Halter and Johnson, 1974). More recently PCB are suggested as the cause for losses of northern pike (Esox lucius) embryos in Michigan hatcheries (Waybrant, 1975). In Sweden Jensen, Johansson, and Olson (1970) suggested a correlation between PCB residues and mortality of salmon eggs and fry.

Multiple Residues

The difficulty of assessing the effects of chemical residues on wild populations of fish is compounded by the simultaneous occurrence of several contaminants. Potentially harmful levels of DDT, dieldrin, PCB, and other toxic chemicals are present in the Great Lakes ecosystem. These multiple residues present analytical uncertainty as well as potential additive effects on aquatic populations.

Prior to 1970 PCB residues in Great Lakes fish were not identified and in many cases were probably mistakenly included in the results given for pesticide residues. The use of gas chromatography-mass spectrometry has improved our ability to identify contaminants, but the procedure remains difficult and expensive. A much more complex problem is the interpretation of the ecological significance or hazards associated with exposure to multiple residues.

Some research indicates a "synergistic" or more-than-additive toxic action between PCB and certain pesticides. Joint action of PCB and DDT was found in chronic exposure tests with *Daphnia magna* (Maki and Johnson, 1975). Toxicity tests with insects have also shown joint action between PCB and some carbonate and organochlorine insecticides (Lichtenstein *et al*., 1969; Plapp, 1973).

Thus, we find that some compounds occur in the environment at concentrations that are known to have adverse effects in laboratory tests. Where these levels are not directly lethal, effects on growth or reproduction may be expressed as slow changes in the size and abundance of fish populations. Therefore, although it appears likely that adverse effects are occurring, it is very difficult to show this conclusively.

Effects on Higher Trophic Levels

Toxic organic chemical residues present a serious hazard to consumer organisms at the higher trophic levels, including man. The biological accumulation of residues and their trophic levels are especially hazardous to animals that utilize fish as a major food source.

Piscivorous birds in the Great Lakes region have suffered unnaturally high mortalities in recent years, and depressed reproduction of some populations has been correlated with chemical residues (Hesse, 1975). The high residue concentrations of PCB in gulls in some regions of the Great Lakes may seriously threaten these bird populations, but the full extent of the problem is unknown.

Even before PCB and pesticide contamination in the Great Lakes was recognized, fur farmers in the region reported reduced reproduction of mink that were fed with Great Lakes fish. Surplus coho salmon or salmon by-products caused death or reproductive failure of mink when these products formed 30% of their diet (Aulerich, Ringer, and Iwamoto, 1973). The relatively high concentrations of DDT, dieldrin, and PCB in the fish were suspected as the causative agents. Subsequent laboratory tests in which mink were fed various doses of DDT and dieldrin in excess of the levels found in the fish did not reproduce the effects. However, 5 ppm PCB added to the experimental diet markedly reduced reproduction, and 15 ppm totally inhibited reproduction and caused death of the adults (Ringer, Aulerich, and Zabik, 1972). These tests established that mink are highly sensitive to PCB toxicity and clearly indicated that residues accumulated in Great Lakes fish were responsible for death and reduced reproduction of commercially reared mink. Because of the high losses resulting from feeding coho salmon, fur farmers have discontinued the use of Great Lakes fish in mink diets.

The residues of PCB in Great Lakes fish pose a potential health hazard to humans. To protect consumers the U.S. Food and Drug Administration has restricted the distribution and sale of fish that contain more than 5 ppm PCB; shipments of such fish from commercial outlets have been confiscated and destroyed. This ruling has curtailed the commercial utilization of most major food fish species in the Great Lakes. Although recreational fisheries are not restricted, state health authorities have warned sport fishermen to limit their consumption of Great Lakes fish. A new information on PCB effects is developed, greater restrictions may be necessary.

NEW TEST PROCEDURES

The problems currently associated with PCB in the Great Lakes are only a single example of the serious impact of synthetic organic chemicals on aquatic ecosystems. Residues of other potentially harmful chemicals (e.g., hexachlorobenzene, the chlorinated napthalenes, pthalate plasticizers) have been found in increasing concentrations in aquatic systems. Clearly there is a need to identify and restrict the distribution of harmful residues before serious damage has occurred.

Industrialized nations throughout the world have a responsibility to develop new strategies for identification and control of harmful chemicals. We can neither afford to wait to study these problems after contamination has occurred, nor can we afford the time and resources to thoroughly investigate each new chemical before it is released to the environment. It is imperative that we develop a systematic approach for evaluation of new materials and new technology. Important new efforts are being made to find correlations between chemical structure and biological activity (Veith and Konasewich, 1975). A chemical classification system based on physical properties, chemical structure, and biological activity would provide some indication of potential hazard. Simple model ecosystems (Metcalf, Sanga, and Kapoor, 1971) and food-chain models (Johnson, 1974) offer additional promise for preliminary testing to identify harmful properties of chemicals.

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SECTION 12

BALANCE OF ORGANIC MATTER IN THE ECOSYSTEM OF THE RYBINSKIY RESERVOIR

V.I. Romanenko[1]

The Rybinskiy reservoir and its drainage basin are located in the southern "taiga" zone, within the boundaries of three districts: Yaroslavskiy, Vologodskiy and Kalininskiy. It was constructed in 1941, and it is one of the largest man-made water bodies in the world with a surface area of 4450 km^2, water volume of 25.4 km^3 and mean depth of 5.6 m. Water inflow, according to data taken over a period of several years, is around 37 km^3/-year. The Volga, Mologa and Shekana River inflows amount to 2/3 of total inflow, with the rest supplied by small rivers.

The reservoir freezes in November and thaws (melts) in April or the beginning of May. According to Secchi disc readings, transparency during the summer is 1-3 m. The content of particles in water is 3-7 mg/liter. The water is of bicarbonate-calcium type according to chemical analysis. The pH is 7.0-7.5, content of organic matter is around 15 mg C/liter, total N is 1.2 mg N_2/liter, total phosphorus is 0.04 mg P/liter, and bicarbonate is 10-20 mg C/liter (monograph "Rybinskiy reservoir and its life", 1972).

Data presented below are based on results of long term observations, for some parameters 5-10 years, up to 20 years for others. Analyses were carried out at 15 day intervals from May through November at six stationary stations distributed along the base area of the reservoir (Figure 1).

INPUT OF ORGANIC MATTER

In the Rybinskiy reservoir, as in all water bodies, there are two basic sources of organic matter: Internal (authocthonous) and external (allochthonous). The photosynthetic production of organic matter by phytoplankton and macrophytic vegetation, which is not large in this reservor (Belavskaya and Kutova, 1963) (approximately 1.8% in the input balance), are the main sources of authocthonous organic matter. Diatoms (Melosira and Aterionella)

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[1] Institute for Biology of the Inland Waters, Academy of Sciences, USSR.

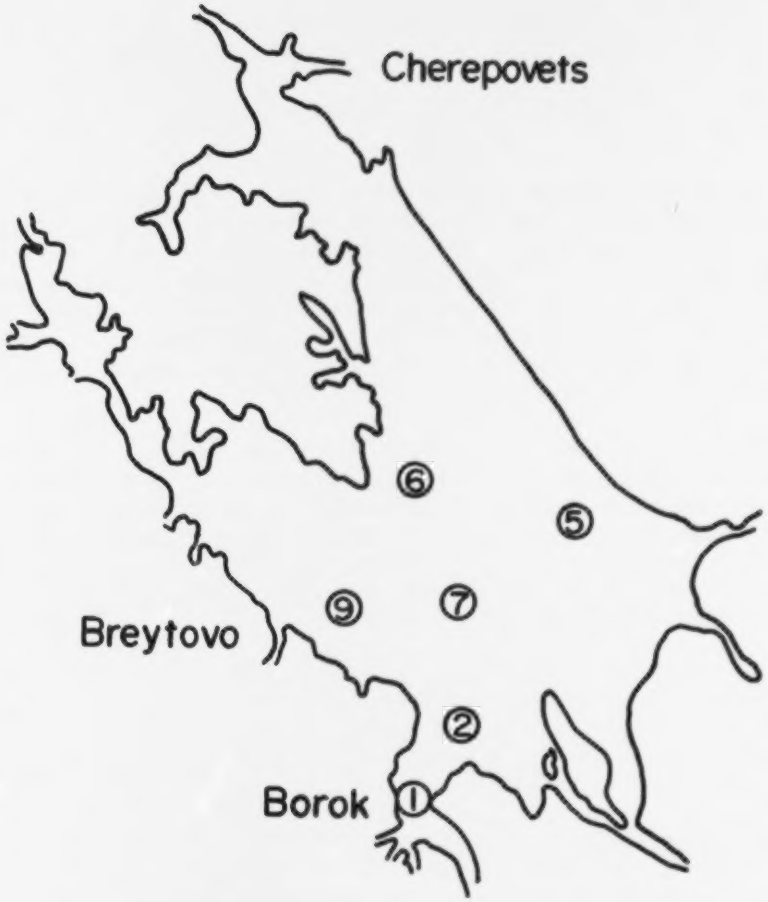
Cherepovets
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Fig. 1. Map-diagram of Rybinskiy reservoir. Figures indicate number and location of standard stations.

are the dominant species of phytoplankton in spring and autumn, but during July and August the blue-green algae are predominant.

Primary productivity of organic matter was determined by the C method (Steeman Nielsen, 1952). The intensity of photosynthesis in the water mass correlated well with water transparency according to Secchi disc readings (Romanenko, 19/3a) according to the formulas:

F_m = $F_i \times 0.7 \times 3\ l \times 1000$, where

F_m = Phytoplankton primary productivity/m^2/24 hours

F_i = Phytoplankton primary productivity in a water sample integrated according to depth, exposed at surface illumination during 24 hours.

l = Secchi disc transparency.

The gross phytoplankton primary productivity (mean for several years) ranged from 100 to 500 thousand ton C for the whole water body; for the surface it changed from 30 to 150 g C/m^2 (Figure 2), with a mean of 76 g C/m^2. Long-term (meaning for several years) fluctuations in the intensity of photosynthesis depended on meterological factors during the year, rate of outflow, and an increase in the reservoir drainage zone. Estuaries of large rivers are the most productive areas. As a rule, the primary productivity is 1.5-2.0 higher than in the central area. In all, 0.05-0.20% energy from solar radiation penetrating the water is used by the algae. Solar radiation is used less effectively during the spring when there are few phytoplankton and the water temperature is low (0.02-0.07%). During the blue-green algae bloom in July and August, given the highest water temperatures, it is used considerably more effectively (0.37%).

By using the data of chemical oxygen demand (COD) and the water balance, the total input of allochthonous organic matter was calculated by Kuznetsev and Bezler (1971). According to their data, 4.75×10^3 ton C as organic matter enters the reservoir with the melted ice and snow. Since this reservoir is located in a low populated region, and the pollution of it as a result of man's activity is not great, the input of organic matter by such sources as wastewaters can be neglected. The input of organic matter due to atmospheric precipitations, in particular in winter with snow on the reservoir surface, is 680 ton C, which is only 0.5% of the value of primary productivity (Romanenko and Bezler, 1971). An additional input of organic matter is also due to rainfall precipitation during summer months. Therefore, it is necessary to consider all these sources of organic matter together, because each of them isolated represents a very small input of allochthonous organic matter.

The total bacterial assimilation of CO_2 and hydrocarbonate (heterotrophic assimilation of CO_2 and chemosynthesis) were determined by the C

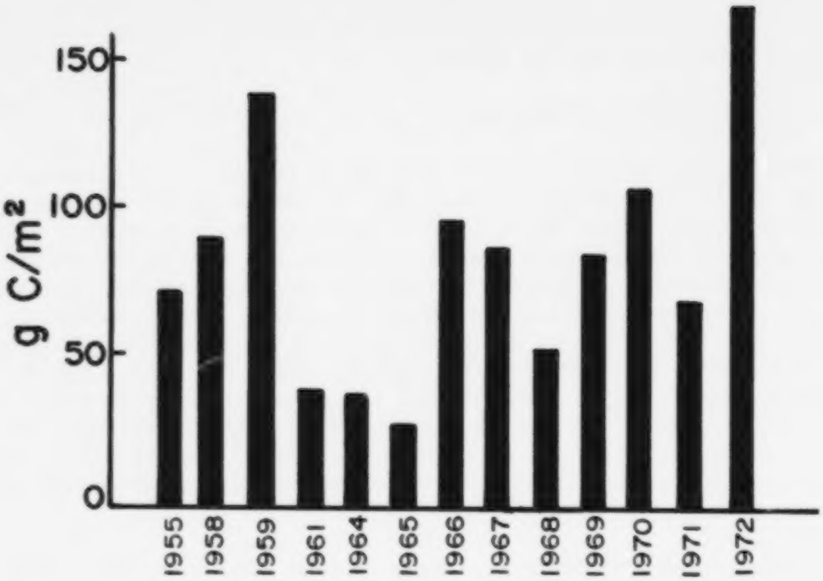

Fig. 2. Average values for phytoplankton production for 13 years in the Rybinskiy reservoir.

method for the whole area of the reservoir during the navigable period. It is not high, (9600 ton C), which means 1.2% of the total organic matter input.

EXPENDITURE OF ORGANIC MATTER

From the very beginning, a part of the organic matter is expended by primary productivity, i.e., by phytoplankton. According to our data, this is equal to 20% of the primary production for 24 hours incubation. When analyzing the destruction of organic matter by the oxygen method in the water mass, the given value is a general sum of the organic matter destroyed by all plankton organisms. Due to the low mean depth and the severe wind regime, the water mass of the Rybinskiy reservoir is very well mixed to the bottom, and the organic matter is mostly oxidized in aerobic conditions by bacterial action. The average number of bacterioplankton by direct counting (Fazumov, 1932) for 20 years of investigation is 1.5 mill/ml and ranged between 0.5-2.5 mill/ml in different years (Figure 3).

By cultivating a sample of bacteria from the reservoir in sterile reservoir water, it is possible to prove that the amount of bacteria determined by direct count is true. In this medium, the bacteria prepared from the water grow very quickly, and it is possible to determine the limits of their development very easily (Romanenko, 1973b).

The bacterial cell volumes in the water are small ($0.3\text{-}0.5\ \mu^3$). The horizontal and vertical distribution of bacterial biomass is uniform in the reservoir, and the total number of bacteria changes little from one point to another. Only near the shores and in the bottom layers is it a little higher. Wet bacterial biomass is equal to 1-2 mg/liter of water.

The generation time, i.e., time needed for the total number of bacteria to double, fluctuated within wide limits and ranged from 5 to 10 hours for individual periods. In the hottest period (July and August) with a doubling in the number of bacteria, generation time is between 16-20 hours; but for temperatures 5-13 C, it can be up to hundreds of hours. For 20 years of investigation, the mean value for the navigable period was 48 hours with temperature fluctuations of 2 to 23 C.

Data on heterotrophic assimilation of CO_2 was used for calculating bacterial biomass (Romanenko, 1964). Its mean value was 35 g C/m^2 or 145 x 10^3 ton C on the whole reservoir (Table 1).

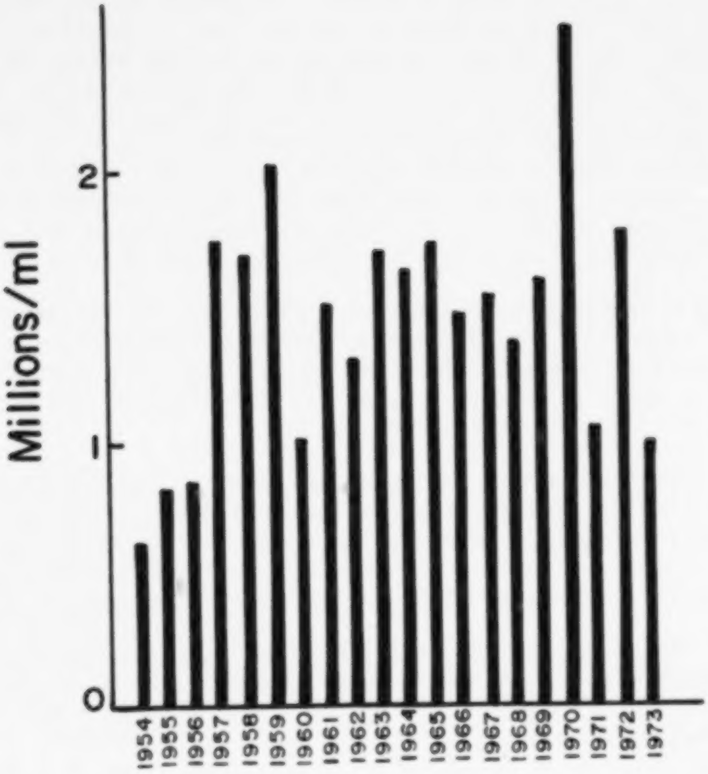

Fig. 3. Average values of the number of bacteria according to data for 2 years of standard observations.

TABLE 1. PRODUCTION OF BACTERIAL BIOMASS IN THE RYBINSKIY RESERVOIR (in C)

	During the navigable period (May-November)				Mean for 24 hours		
Year	Number of days	In 1 liter, mg	Under 1 mg^2, g	For the whole reservoir ton	In 1 liter, mg	Under 1 mg^2, g	For the whole reservoir ton
1964	140	6.7	33	117000	0.047	0.24	836
1965	158	10.6	55	231000	0.067	0.35	1460
1966	151	7.4	39	174000	0.049	0.26	1150
1967	150	5.3	28	117000	0.035	0.19	780
1968	187	3.6	21	86000	0.019	0.11	460
Mean	157	6.7	35	145000	0.043	0.23	937

A very strong purification effect is produced by this bacterial biomass. A great amount of reduced organic compounds is oxidized through this effect, where the compounds are oxidized by oxygen in enzymatic reactions. According to the data, in essence we did not observe a significant increase in bacterial biomass. This total value for a large amount of time and for each short time interval is not very great.

The destruction of organic matter was determined on the basis of oxygen consumption in dark bottles during the incubation of samples for 24 hours at ambient water temperature (Table 2). From this table, it is possible to see that this value for the whole reservoir for the navigable period ranged from 270,000 to 950,000 ton C of organic matter decomposed, with a mean value of 550,000 ton C for 5 years of observations (129 g C/m^2) and a fluctuation between 64-214 g C/m^2 in different years.

TABLE 2. DESTRUCTION OF ORGANIC MATTER IN THE WATER (in C)

	During the navigable period (May-November)				Mean for 24 hours		
Year	Number of days	In 1 liter, mg	Under 1 mg^2, g	For the whole reservoir, ton	In 1 liter, mg	Under 1 mg^2, g	For the whole reservoir ton
1958	135	18	101	450000	0.13	0.75	3340
1965	162	20	116	480000	0.12	0.72	2960
1966	167	40	214	950000	0.24	1.28	5670
1967	154	28	150	633000	0.18	0.97	4110
1968	194	11	64	270000	0.06	0.33	1390
Mean	162	23	129	556000	0.14	0.91	3494

If we take into account that the rest of the population destroys only around 20% of the total organic matter (especially algae and invertebrates), it is easy to calculate the correlation between bacterial biomass and destruction. The production of bacterial biomass was 30% of the amount of destroyed organic matter (expressed as carbon). By means of pure cultures it was demonstrated that the correlation between these data (K_1 coefficient) ranged from 25-35%, which proves the reality of our calculation of bacterial production by means of the data on heterotrophic assimilation of CO_2.

Also in the bottom sediments a great amount of organic matter is decomposed in aerobic or anaerobic conditions according to the redox potential through the activity of aerobic or anaerobic organisms or both. In this reservoir, the bottom layers are very well supplied with dissolved oxygen, and oxygen deficits are only observed in the flood areas in which the rH_2 fluctuates from 10-12, and sulfate reduction and methane formation processes are most intense. The rH_2 is 10-17 in the peat slimes and 5-20 in the sandy ones with small amount of debris. In such conditions aerobic destruction of organic matter prevails.

The mean number of bacteria by direct count is 0.5-30 billion/g of wet slime. Fifteen years ago, when the amount of organic matter from the recently flooded areas was high enough, a very strong production of methane gas derived from the activity of methane-producing bacteria was observed and, especially in winter season, the big bubbles of this gas induced the death of fish by asphyxiation. The methane formation process at this time has sharply decreased.

To determine the destruction of organic matter in the bottom sediments, the oxygen consumption of a column of slime isolated in a glass tube (Hayes and MacAulay, 1959) was used, and the balance of CO_2 extracted from the slime into the water (Romanenko and Romanenko, 1969) was employed to determine the aerobic decompositon of organic matter.

The results showed that 74,000 ton C as organic matter are decomposed in the slime by aerobic processes during the navigable period, which means 20 g C/m^2. Nearly 10 g C/m^2 are decomposed in anaerobic conditions. This means that 23% of the organic matter is destroyed in the water mass.

The loss of organic matter through the lower outlet was calculated according to oxidizability and water balance as 179,000 ton C by Kuznetsov and Bezler (1971).

For the balance (Table 3) of the input and expenditure of organic matter in the ecosystem of the Rybinskiy reservoir, taking into account only the most important parameters, we can say that as a whole, in this ecosystem, the input of organic matter is 826,000 ton C or 199.9 g C/m^2.

By bacterial destruction or outlow through the outlet, the expenditure of organic matter is 838,000 ton C, so the difference is -8400 ton C. If we discount the loss of organic matter through the outlet (837,000 - 179,000), it is possible to see that the purification effect due to the

TABLE 3. MAIN ELEMENTS OF THE BALANCE OF ORGANIC MATTER IN THE RYBINSKIY RESERVOIR

Element	For the whole reservoir x 10^3, ton C	Under 1 m^2, g C	%	Note
Input				
Gross production of phytoplankton	330	78	39	Mean for 11 years (from 1955 to 1970) During 1956, 57, 62, and 63 the analyses were not done
Production of macrophytic vegetation	2 14	3.5	1.8	According to data for 1965 (Belavskaya and Kutova, 1966)
Bacterial assimilation of CO_2	9.6	2.4	1.2	Mean for 5 years (1964-1969)
Input of organic matter from the watershed and atmospheric precipitations	475	116	58	According to data for 1965
Total Expenditure				
Destruction in water in summer	516	129	62	Mean for 5 years (1958,1965,1968)
Destruction in water in winter	39	9	4.3	According to data for 1965
Aerobic destruction in slime in summer	84	21	10	Mean for 2 years (1967, 1968)
Aerobic destruction in slime in winter	19	5	2.4	Calculated according to the coefficient of Van Hoff (K = 3)
Outflow of organic matter through the outlet	179	44.7	21	According to data for 1965
Total	837	208.7	100	
Difference between input and expenditure of organic matter	-8.4	-8.8	-	

activity of the population of water and slime (fundamentally microorganisms) is 658,000 ton C as organic matter.

Of course, we know that the accuracy of the data depends on the analytical possibilities, and because of this we are inclined to examine such a good agreement between the input and expenditure of organic mater as possibly affected by some random element. Nevertheless, the main sources of input and expenditure of organic matter in such a large and complex man-made reservoir as the Rybinskiy reservoir are clear.

Only a very small amount of matter and energy, in comparison with the amount involved in the fundamental processes, flows through the rest of the population of the reservoir. Taking into account the data from the biomass and cycles of life of the different animals (Yu.I. Sorokin, see monograph "Rybinskiy reservoir and its life," 1972), in the second trophic level (herbivorous, detritous protozoa, rotatoria and crustacea) nearly 20% of the incoming organic matter is utilized. In the third trophic level (predator zooplankton, zoobenthos and fish larvae) only 0.2% is used and at least as much for forage and predator fish. For man, it reaches only 0.05%. Of course, it is necessary to remember that the last values expressed as absolute units are fairly inexact because the calculation of such parameters as species composition, zooplankton biomass and life cycles are only approximated. So, as useful products on man's table, he receives only tenths or hundreths of parts of the initial input of organic matter, and a large part of it is utilized by bacteria.

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SECTION 13

THE IMPORTANCE OF TROPHIC BONDS IN THE BACTERIAL DESTRUCTION OF ORGANIC MATTER

P.P. Umorin[1]

In connection with the problem of purifying industrial and domestic wastewaters and ascertaining the role of bacteria as a main factor in purifying the dissolved organic matter (DOM), it is necessary to show the role of organisms that feed on bacteria during the process. Unfortunately, work carried out in this field and concerned with the role of the protozoa is contradictory (Kryuchkoya, 1968). In the experiments of a great number of authors (Butterfield, Purdy, Theriault, 1931; Phelps, 1953; Javorinsky, Prokesova, 1963; Nikolyuk, 1965; Straskrabova-Prokesova, Legner, 1966; Jensen, Ball, 1970), the oxygen consumption and nitrogen fixation were more intensive in mixed cultures of bacteria and protozoa than in pure cultures of bacteria. The results of these and other authors serve as a foundation for the hypothesis first put forward by Butterfield (Butterfield, Purdy, Theriault, 1931) that organisms preying upon bacteria keep the latter in a state of continuous reproduction or physiological "youth" by a simple decrease of their number. This should facilitate a greater rate of decomposition of organic matter. The above-mentioned authors, however, do not analyze the correlation between the actual conditions of the experiment and its results. In addition, the role of separate species and the quantitative characteristics of the bacteria and protozoa relationships are far from clear. This is especially true of the colourless flagellates. Their role in the life of water bodies has almost not been studied. The aim of this work is to study the rate of organic matter decomposition with bacteria and protozoa in continuous cultures, maximizing the similarity to natural conditions.

METHODS

All the experiments have been performed using a continuous culture device described previously (Umorin, 1975) with various dilution rates (D). Pratt's solution served as the nutrient medium. Phenol at a concentration of 25 mg/liter or glucose at a concentration of 50 mg/liter was added to the solution as the only source of organic carbon. Before the experiments, the reactor, 1.2 liter in volume, was filled with the nutritive medium and innoculated with bacteria taken from a water-body and previously kept in a continuous culture on the Pratt medium with an organic matter (phenol or glucose) used in the experiment. Then a continuous run was started with the required dilution rate. When a steady state (constant concentration of organic matter and constant number of bacteria) was reached, protozoa were added to the reactor and the experiment was continued until a new steady state was attained. Thus, the first part of the experiment (organic matter + bacteria) served as a control for the second part (organic matter + bacteria + protozoa). As for the protozoa, the infusoria ciliates Paramecium caudatum, Ehrb. were used with the experiments with phenol, and the zooflagellates Pleuromonas jaculans, Perty, in the experiments with glucose.

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[1] The Institute of Biology of Inland Waters of the Acad. Sci. USSR.

To clarify the interrelations of bacteria and protozoa in a medium deficient in nutrients, experiments have also been performed with infusoria ciliates P. caudatum in Pratt solution in which the concentration of nitrate nitrogen was diminished to 1 mg/liter. All the experiments were conducted at a temperature of 22 C. Altogether, the following experimental variables have been used:

1. Bacteria on complete Pratt medium with phenol at D = 0.02 hrs^{-1}.
2. Bacteria and infusoria on complete Pratt medium with phenol at D = 0.02 hrs^{-1}.
3. Bacteria on complete Pratt medium with phenol at D = 0.04 hrs^{-1}.
4. Bacteria and infusoria on complete Pratt medium with phenol at D = 0.04 hrs^{-1}.
5. Bacteria on pratt medium given deficient nitrogen with phenol at D = 0.02 hrs^{-1}.
6. Bacteria and infusoria on Pratt medium given deficient nitrogen with phenol at D = 0.02 hrs^{-1}.
7. Bacteria on complete Pratt medium with glucose at D = 0.06 hrs^{-1}.
8. Bacteria and flagellates on complete Pratt medium with glucose at D = 0.06 hrs^{-1}.
9. Bacteria on complete Pratt medium with glucose at D = 0.08 hrs^{-1}.
10. Bacteria and flagellates on complete Pratt medium with glucose at D = 0.08 hrs^{-1}.

All the variables of the experiment were conducted with three replications. Every day the numbers of bacteria and flagellates were determined in the reactor by direct counts in the Goryavev chamber at microscope magnification of 900x for bacteria and 150x for flagellates. Infusoria were counted in the Bogorov chamber under a MBS-1 microscope. To see the balance of organic matter, the numbers of organisms were expressed in dry weights in mg/liter. The latter were calculated from their biovolume; specific gravity of the wet biomass was accepted as an equal unit and the dry weight - 15% of the biomass. The mean volume of bacteria developing on the medium with phenol was 1 μ^3, and on the medium with glucose 0.5 μ^3; in this case, 1 mg of dry weight of bacteria per liter is equivalent to 7.5 million and 15 million cells per milliliter, respectively. With the mean volume of P. jaculans 25 μ^3, 1 mg of dry weight per liter corresponds to the number 300 thousand cells per ml of this flagellate. The dry weight of one infusorian P. caudatum was taken from G.G. Vinberg's work (Vinberg, 1949), 0.08 x 10^{-3} mg. Then their dry weight of 1 mg/liter is equivalent to the number 12.5 cells/ml. Concurrently with a calculation of the number of organisms, the phenol concentration was determined by pyramidone method (Lurie, Rybnikova, 1966), and that of glucose by a reagent with phenol (Bikbulatov, Skopintsev, 1974).

RESULTS

In all the experimentally conditions, a steady component value was established in the reactor, according to the type of damped oscillations in experiments 2, 4, and 6, and aperiodically in the remaining ones. On the complete Pratt media, the concentration of the organic matter in the reactor was established at a higher level in the experiments with protozoa than without them. This indicates that when mineral nutrients are provided and the growth rate of bacteria is limited only by the concentration of organic matter, the bacteria alone decompose it faster than in the mixed cultures with protozoa. Since the number of bacteria in the presence of protozoa was significantly lower than without them, the decrease in the rate of decomposition of organic matter may be explained by the decrease in the number of bacteria due to predation by protozoa. Nevertheless, in the experiments with nitrogen deficiency, destruction of organic matter proceeded faster in the presence of protozoa, in spite of the decrease in the number of bacteria; i.e., one and the same factor, protozoa, may either accelerate or slow down destruction of organic matter depending upon the condition.

TABLE 1. VALUES OF THE COMPONENTS IN MG/LITER (DRY WEIGHT) AT THE STEADY STATE (AVERAGE THREE REPETITIONS).

Experiment number	D hrs	Organic Matter	Bacteria	Protozoa	Time of establishing steady state (days)
1	0.02	4.9	11.1	-	8
2	0.02	19.6	0.36	1.2	36
3	0.04	10.4	7.5	-	8
4	0.04	20.6	1.6	0.8	24
5	0.02	23.2	0.89	-	30
6	0.02	21.5	0.83	0.39	30
7	0.06	6.7	12.2	-	4
8	0.06	11.0	6.3	1.9	25
9	0.08	8.9	11.5	-	4
10	0.08	9.5	10.6	0.3	25

To reveal the character and quantitative aspect of interrelations between bacteria and protozoa in the process of destroying organic matter, it is necessary to use mathematic models. We used models similar to Canale's (1970) models, in which the dependences between specific growth rates and feeding of organisms and the concentration of their food was linear. Such a linearization has appeared to be quite acceptable for test conditions allowing only a small change interval in the component values.

For the system--organic matter-bacteria:

$$\left.\begin{aligned} \frac{dS}{dt} &= D(S_1-S) - k_1SH \\ \frac{dH}{dt} &= k_2SH - DH \end{aligned}\right\} \quad (1)$$

For the system--organic matter-bacteria-infusoria

$$\left.\begin{aligned} \frac{dS}{dt} &= D(S_1-S) - k_1SH - k_3SP \\ \frac{dH}{dt} &= k_2SH - DH - k_4HP \\ \frac{dP}{dt} &= k_5HP + k_6SP - DP \end{aligned}\right\} \quad (2)$$

where S - is the concentration of organic matter in the reactor

S_1 - the same, in the inflowing medium

H - concentration of bacteria in dry matter

P - the same, for protozoa

D - dilution rate

t - time

k_i - coefficients of proportinality.

For the system--organic matter-bacteria-zooflagellates, a model was constructed taking into account the possibility of feeding the zooflagellates with the dissolved organic matter (DOM) (Goryacheva, 1975):

$$\left.\begin{aligned}\frac{dS}{dt} &= D(S_1 - S) - k_1SH - k_3SP \\ \frac{dH}{dt} &= k_2SH - DH - k_4HP \\ \frac{dP}{dt} &= k_5HP + k_6SP - DP\end{aligned}\right\} (3)$$

Here the term k SP is the consumption of DOM by zooflagellates and k_6SP is their growth (per unit time) due to DOM consumption.

The mathematical models (1) - (3) are not applicable for experiments with nitrogen deficiency, since in this case, the concentration of organic matter is not the factor limiting the growth of bacteria. A system of equations which consider the role of nitrogen as the limiting factor has been made up.

For the system--organic matter-bacteria:

$$\left.\begin{aligned}\frac{dN}{dt} &= D(N_1 - N) - k_1NH \\ \frac{dH}{dt} &= k_2NH - DH \\ \frac{dS}{dt} &= D(S_1 - S) - k_3NH\end{aligned}\right\} (4)$$

For the system--organic-matter-bacteria-protozoa:

$$\left.\begin{aligned}\frac{dN}{dt} &= D(N_1 - N) - k_1NH + k_4P \\ \frac{dH}{dt} &= k_2NH - DH - k_5HP \\ \frac{dP}{dt} &= k_6PH - DP \\ \frac{dS}{dt} &= D(S_1 - S) - k_3NH\end{aligned}\right\} (5)$$

where N_1 is the concentration of nitrogen in the inflowing medium, N is the concentration of nitrogen in the reactor, and the remaining designations are the same as in models (1) - (3). In models (4) and (5), the term $D(N_1-N)$ shows the influx of nitrogen into the reactor; k_1NH is the net consumption of nitrogen by bacteria (difference between consumption and excretion); k_4P is the excretion of nitrogen by protozoa; k_2NH and k_3NH are the growth of bacteria and the consumption of organic matter by the bacteria per unit time.

To determine the rates of feeding and reproduction of the living components, it is necessary to calculate the K_i coefficients. The coefficient k_1 and k_2 were calculated by substituting into model (1) the steady-state values of the components and equating the right members of the equations to zero. For the experiments with phenol, they appeared to be $k_1 = 0.008$ and $k_2 = 0.004$; for the experiments with glucose, $k_1 = 0.032$ and $k_2 = 0.009$[1]. Coefficients k_4 and k_5 of model (2) and k_3 and k_4 of model (3) were calculated by introducing into these systems the coefficients k_1 and k_2 from model (1). They were for model (2) $k_4 = 0.051$, $k_5 = 0.024$; for model (3) $k_4 = 0.02$. Coefficients k_5 of model (3) was determined using Y (yield coefficient) taken from our previous work on feeding of the zooflagellate *P. jaculans* on bacteria (Umorin, 1975). That coefficient was equal to 33%. Since $k_5/k_4 \times 100\% = k_1$, then $k_5 = 0.006$. Coefficient k_6 was calculated from the third equation of model (3). It appeared to be equal to 0.002.

Since in the experiments the concentration of nitrogen with its deficiency was not determined, coefficients k_2 and k_3 of model (4) were taken from another experiment which had been performed to evaluate the dependence of the specific growth rate of bacteria μ upon the source concentration of nitrate nitrogen (N). Phenol at a concentration of 25 mg/liter was used as the source of organic carbon. The results of this experiment (average 6 repetitions) are provided in Table 2.

TABLE 2. DESTRUCTION OF PHENOL WHEN LIMITED BY NITROGEN

Initial concentration of nitrate nitrogen mg/liter	Specific growth rate of bacteria, hrs^{-1}	Initial biomass of bacteria mg/liter of dry weight	Growth of bacteria in dry weight for 10 hrs, mg/liter	Phenol consumed for 10 hrs, mg/liter
0.5	0.016	0.8	0.138	0.24
1.0	0.021	0.8	0.176	0.39
1.5	0.027	0.8	0.248	0.54

Judging by the amount of bacterial growth for a period of 10 hrs,, the nitrogen concentration in the experiments decreased by a negligible value

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[1] All the k_i coefficients have a dimension of liter-mg^{-1}hrs^{-1}.

(parts per hundred mg/liter) and may be considered constant during this time period. The dependence described in the experiments μ on N may be approximated by the linear function $\mu = 0.023$ N as shown in Figure 11, i.e., $k_2 = 0.023$. The quantity of the consumed phenol, according to the data given above, is approximately twice as great as the growth of bacteria; therefore, we accepted $k_3 = 2 k_2$. The coefficient k_1 was chosen so that model (4) would most closely simulate the experimental data; it was taken to be 0.0028. The values of coefficients k_2 and k_3 of model (4) do not suit model (5). The coefficients k_1, k_2, k_3, and k_4 for model (5) were chosen, preserving their ratios, as in model (4), in such a way that mathematical model (5) would most closely approach the results of experiment 6; in this case, coefficients k_5 and k_6 were taken as equal to k_4 and k_5 of model (2). They were accepted to be $k_1 = 0.0055$, $k_2 = 0.0438$, $k_3 = 0.0876$, $k_4 = 0.0025$, $k_5 = 0.0510$, and $k_6 = 0.0240$.

After determining or while choosing the coefficients, the processes described by the models were computed on a "Minsk-22" digital computer to compare the calculated and experimental curves. The calculated and experimental curves correspond quite well, that is the mathematical models given above describe rather correctly the processes taking place in the experiments. An analysis of the models allows us to obtain quantitative data on the growth and feeding rates of the living components and on the character of their interrelationships in the process of organic matter decomposition. The product k_4H in model (2) is the rate of bacteria consumption by unit dry weight of infusoria. Its calculation and conversion for the number of cells show that one infusorian P. caudatum consumes about 50 thousand bacterial cells per hour at $D = 0.04$ hrs^{-1}, and 25 thousand at $D = 0.02$ hr^{-1} at the steady state in the reactor.

Analysis of models (4) and (5) renders it possible to understand why the destruction of organic matter by bacteria accelerates in the presence of infusoria given a nitrogen deficiency. The necessity to make the coefficients k_2 and k_3 in model (5) almost twice as great as those in model (4) indicates that in the former case nitrogen was more easily assimilable as present in the reactor, than nitrate nitrogen inflowing with the medium. As is known, some of the substances excreted by infusoria are urea and uric acid (Dogel, 1951). The infusoria apparently play a role in stimulating the decomposition of organic matter by creating nitrogen circulation and liberating it in a form more readily accepted by the bacteria in the environment. In model (3) the values of k_3S and k_4H are correspondingly the values of glucose and bacteria consumption by zooflagellates. Calculation of them has shown that at steady state with a dilution rate of $D = 0.06$ hrs^{-1}, 0.126 mg of dry weight of bacteria and 0.055 mg of glucose are consumed per hour by 1 mg dry weight of zooflagellates. At a dilution rate of $D = 0.08$ hrs^{-1} these values are equal to 0.212 and 0.047 mg, respectively.

In such a manner, in test conditions, from one-sixth to one-third of the zooflagellates' food consumption is satisfied by consumption of organic matter. To verify the fact that the zooflagellate P. jaculans feeds on dissolved organic matter, model (3) was calculated by computer, with coefficients k_3 and k_6 equal to zero, so that an exclusion of glucose from the zooflagellate's ration was indicated. The calculation indicated that at a dilution rate of $D = 0.08\ hrs^{-1}$ in this case, a washing out of the zooflagellates from the reactor takes place (factually not observed), but at $D = 0.06\ hrs^{-1}$, the estimated calculated curve does not correspond to the experimental one.

When converting the dry weight of bacteria consumed into the number of cells obtained we found that one zooflagellate consumed 6.3 and 9.7 bacteria per hour.

Assuming that the coefficients of feeding and reproduction of the organisms obtained in the experiments are close to those in nature, we can estimate the degree of participation of bacteria, zooflagellates and infusoria in the processes of destruction and transformation of organic matter in any water body, e.g., in the Rybinskoye reservoir. In this water body the concentration of an easily degradable organic matter is on the average 10 mg/liter (Skopintsev, Bakulina, 1966); the biomass and the production of bacteria biomass are 0.8 mg/liter and 0.6 mg/liter day (Sorokin, 1971); the biomass of the zooflagellates is up to 0.02 mg/liter (Zhukov, 1973); and the biomass of infusoria is about 1 mg/liter (Mamayeva, 1971). Using these data and the calculated coefficients, we find that bacteria consume from 250 to 750 mg of DOM per day in a cubic meter. The growth in their dry weight must make up from 100 to 250 mg/m^3 per day which quite corresponds to the above mentioned value of the production of wet biomass.

Infusoria consume about 360 mg/m^3 of the bacterial biomass per day, or about a half their daily production. In one cubic meter, the zooflagellates consume daily 4.8 mg of DOM and 1.3 mg of bacterial biomass, i.e., they continue to feed in nature mostly on DOM, consuming only 0.2% of the bacterial production as food.

Thus, bacteria are the main consumer of organic matter in water bodies. Zooflagellates cannot affect the rate of decomposition of organic matter by predation on bacteria or by direct consumption of dissolved organic matter. As for the infusoria, they can notably slow down the decomposition or organic matter by bacteria by means of reducing their number due to predation. However, in water bodies, as a rule, a deficiency in nutrients has been observed. Guaranteeing their recycling, infusoria may accelerate the bacterial destruction of organic matter.

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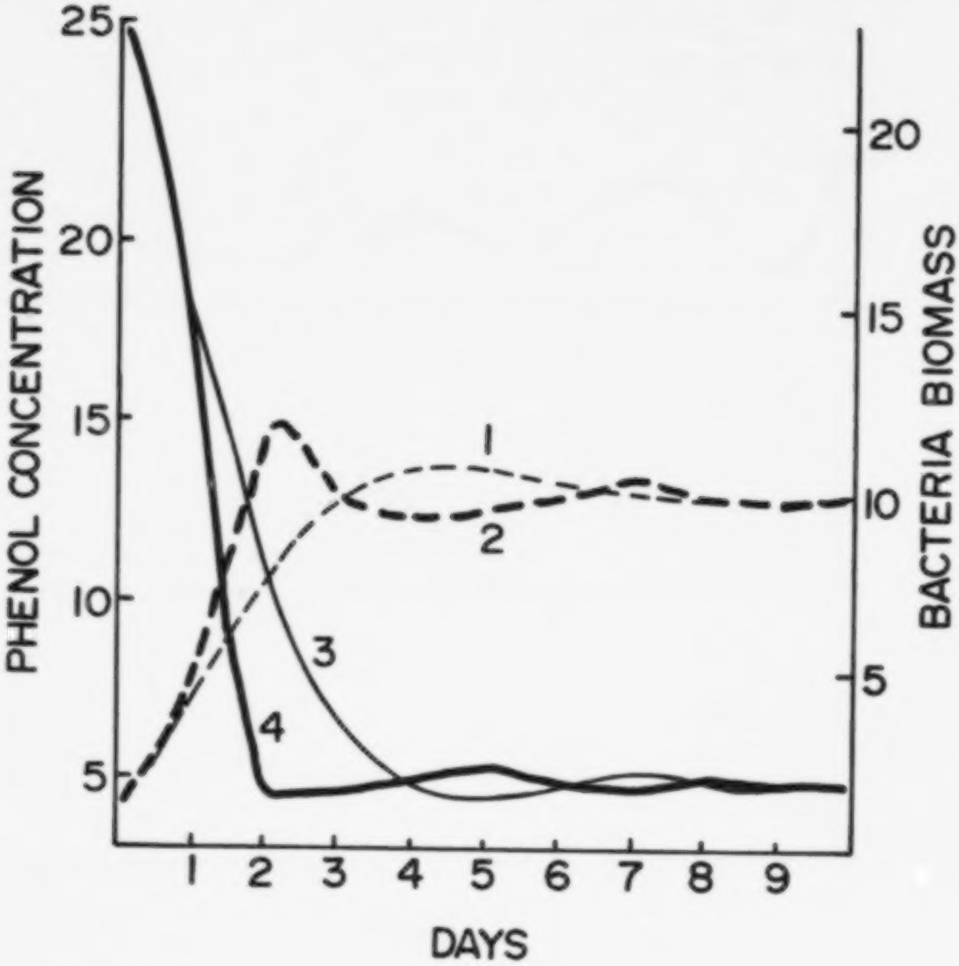

Fig. 1. Change of component values in time -- experiment 1. Left vertical axis - concentration of organic matter mg/L, - right - bacteria biomass in mg/L of dry weight. 1 - Bacteria biomass, calculated curve; 2 - the same, experimental curve; 3 - concentration of organic matter, calculated curve; 4 - same, experimental curve.

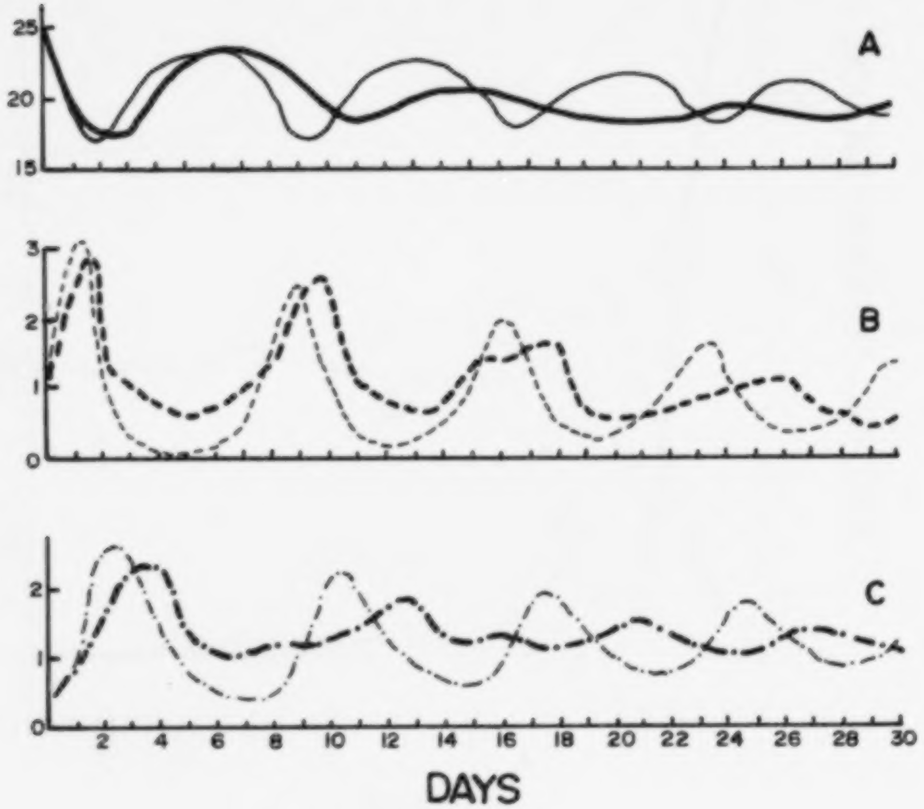

Fig. 2. Change in component values in time -- experiment 2. Vertical axis: A - Phenol concentration mg/l, B - bacteria biomass in mg/l of dry weight, C - infusoria biomass - in mg/l of dry weight. 1 - calculated estimated curves, 2 - experimental curves.

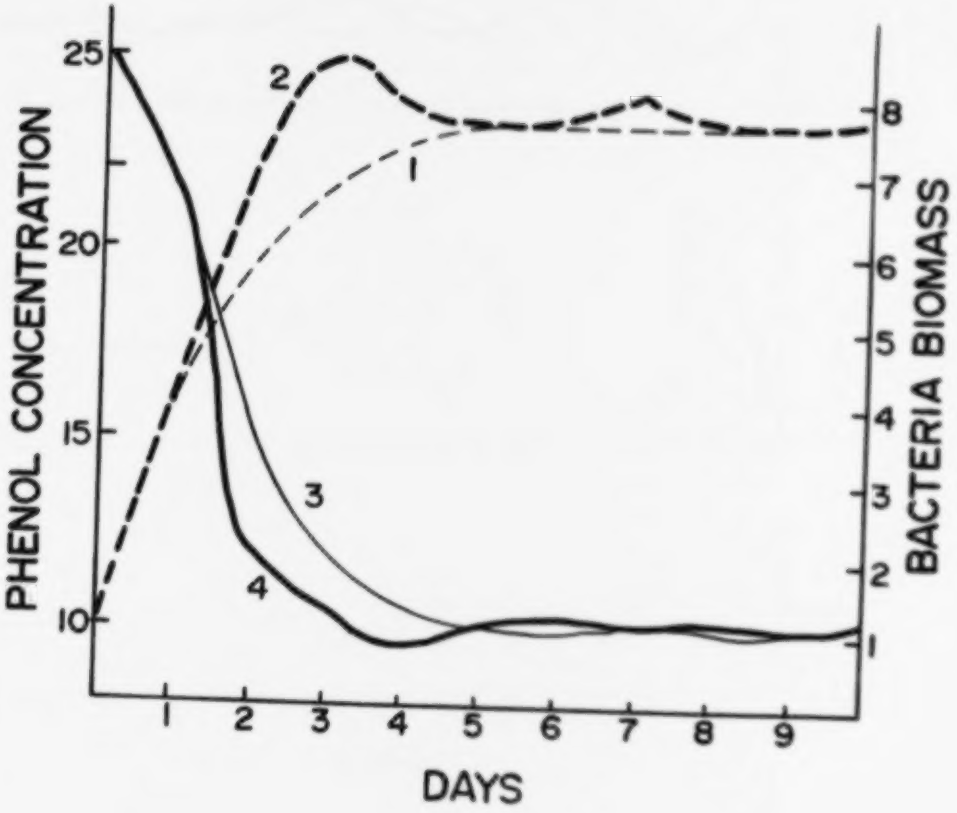

Fig. 3. Change in component values in time -- experiment 3. Symbols are the same as for figure 1.

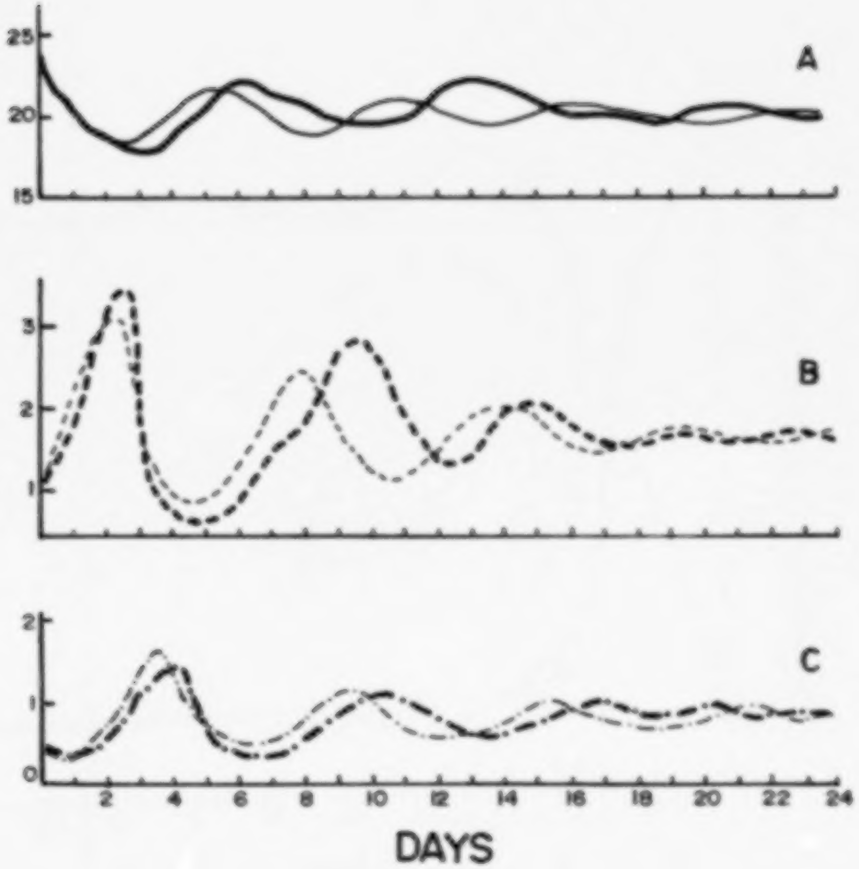

Fig. 4. Change in component values in time -- experiment 4. Symbols are the same as for figure 2.

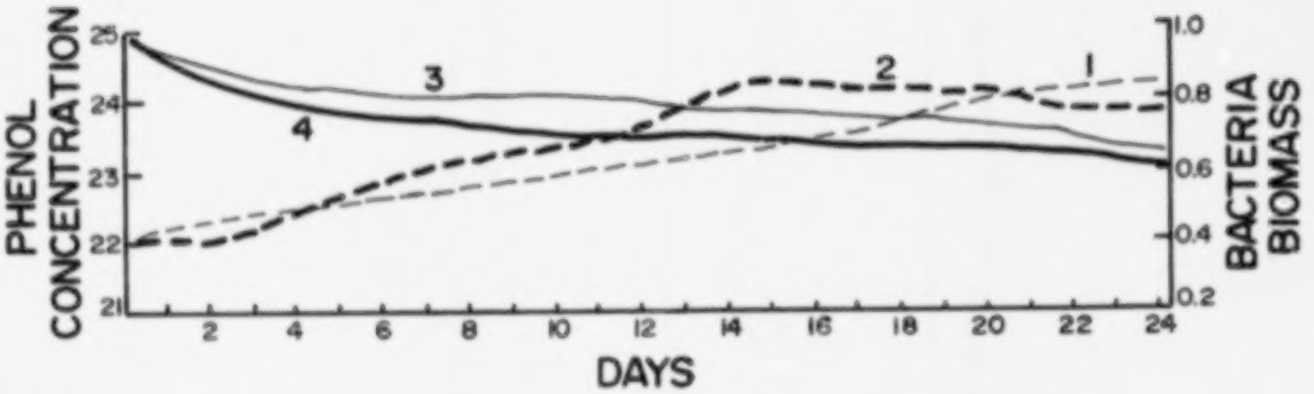

Fig. 5. Change in component values in time -- experiment 5. Symbols are the same as for figures 1, 3.

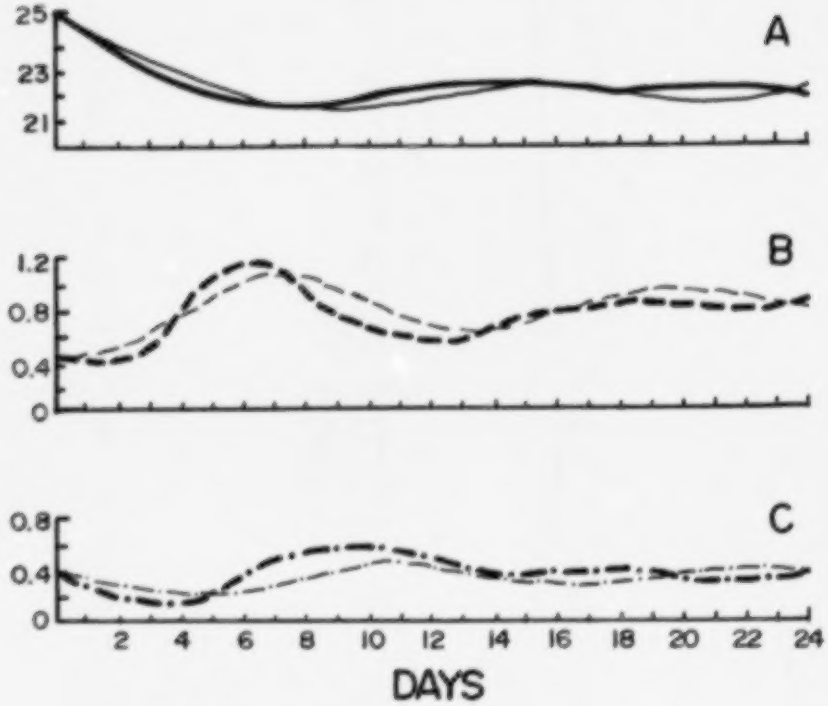

Fig. 6. Change in component values in time -- experiment 6. Symbols are the same as for figures 2, 4.

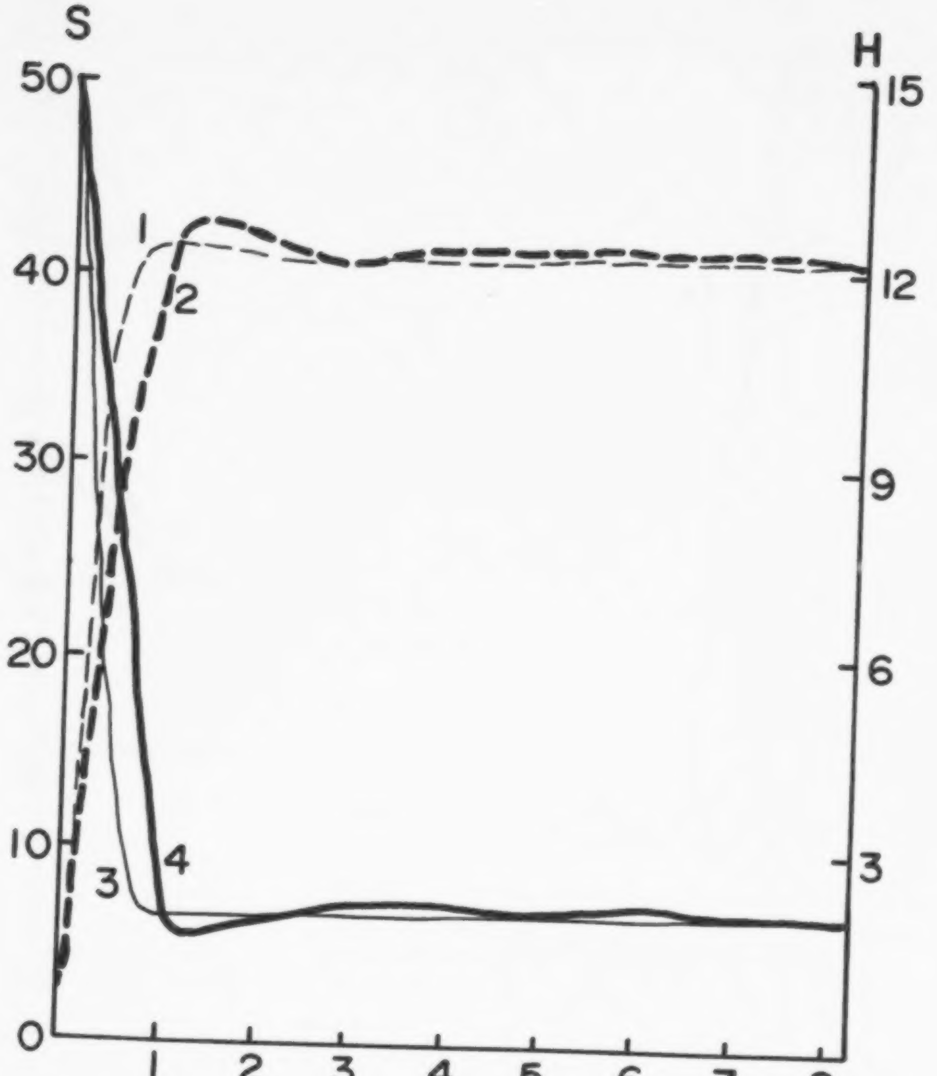

Fig. 7. Change in component values in time -- experiment 7. Symbols are the same as figures 1, 3, 5.

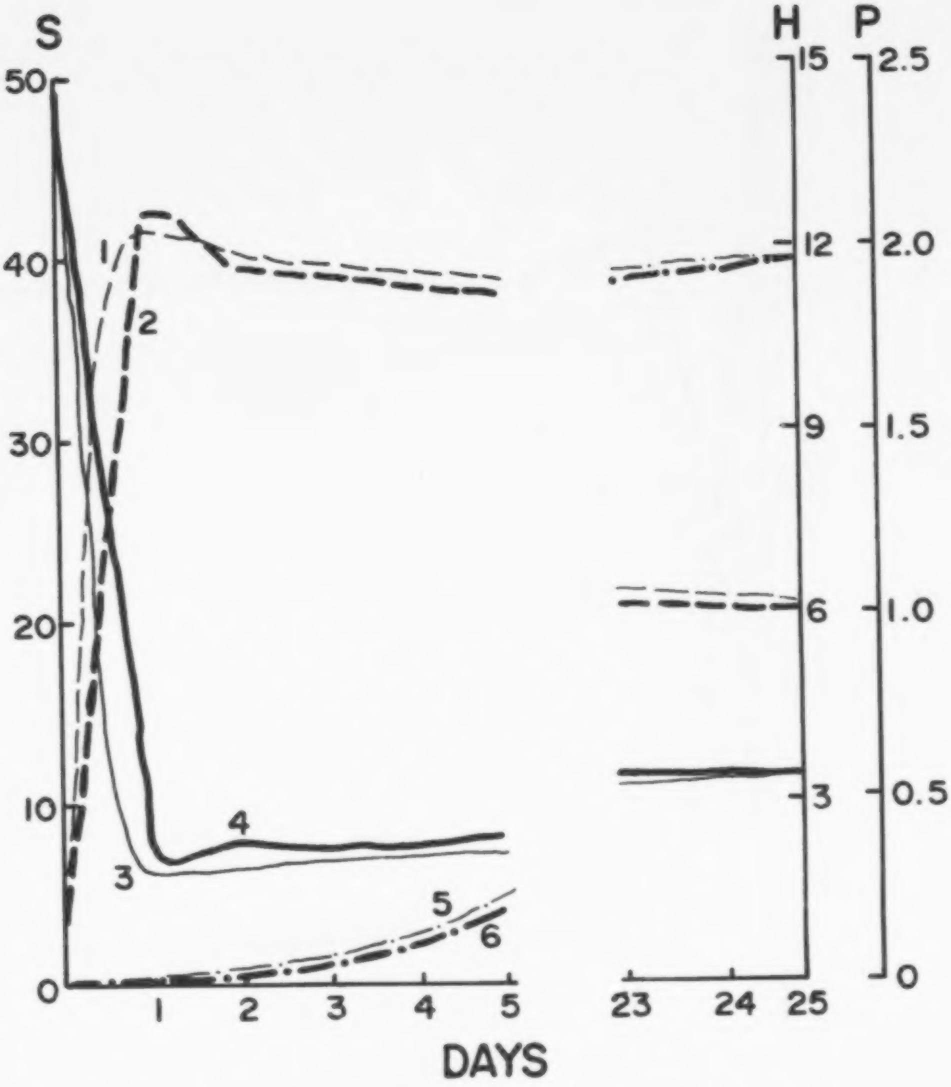

Fig. 8. Change in component values in time -- experiment 8. Vertical axis: S - glucose concentration mg/l; H - bacteria biomass in mg/l of dry weight; P - zooflagellate biomass in mg/l dry weight. 1 - bacteria biomass, calculated estimated curve; 2 - same, experimental curve; 3 - glucose concentration, calculated estimated curve; 4 - same, experimental curve; 5 - zooflagellate biomass, calculated estimated curve; 6 - same, experimental curve.

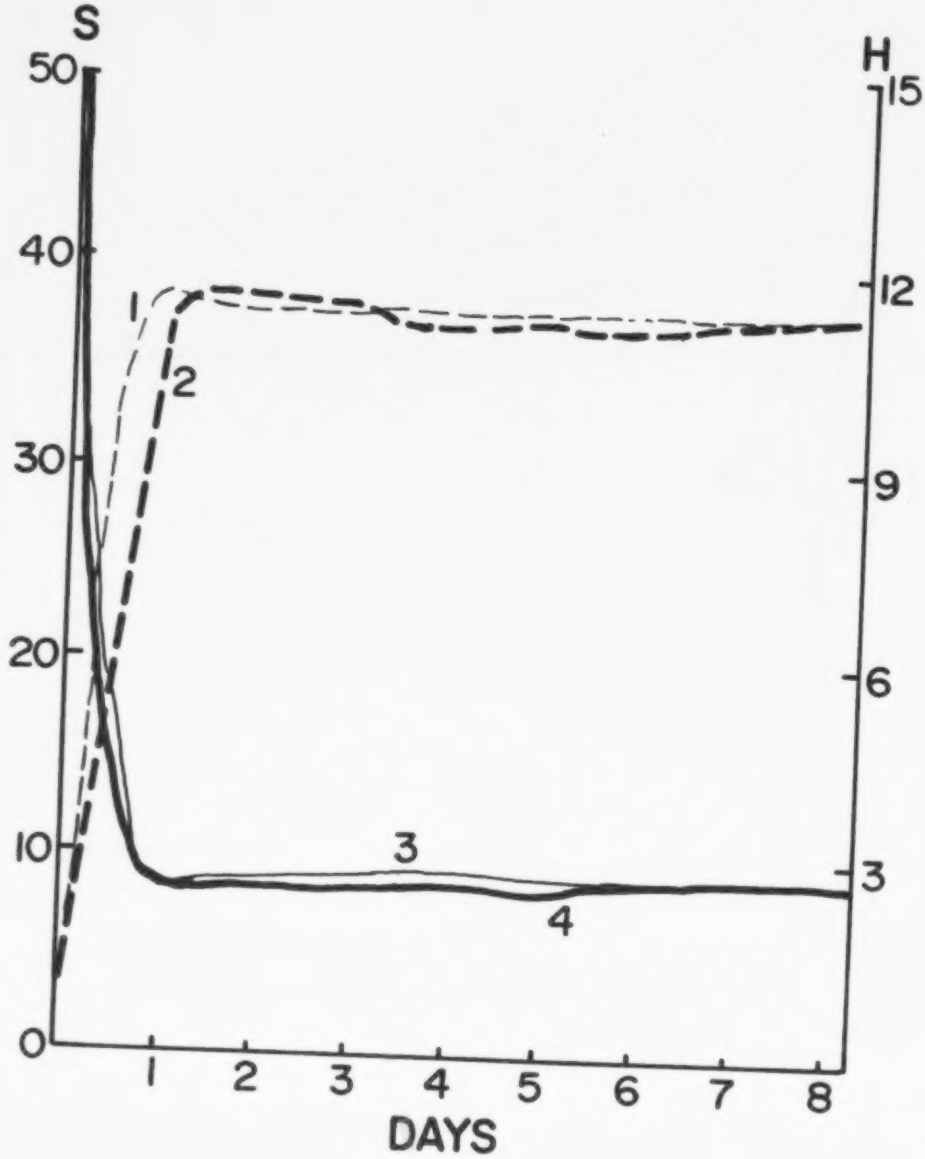

Fig. 9. Change in component values in time -- experiment 9. Symbols are the same as for figures 1, 3, 5, 7.

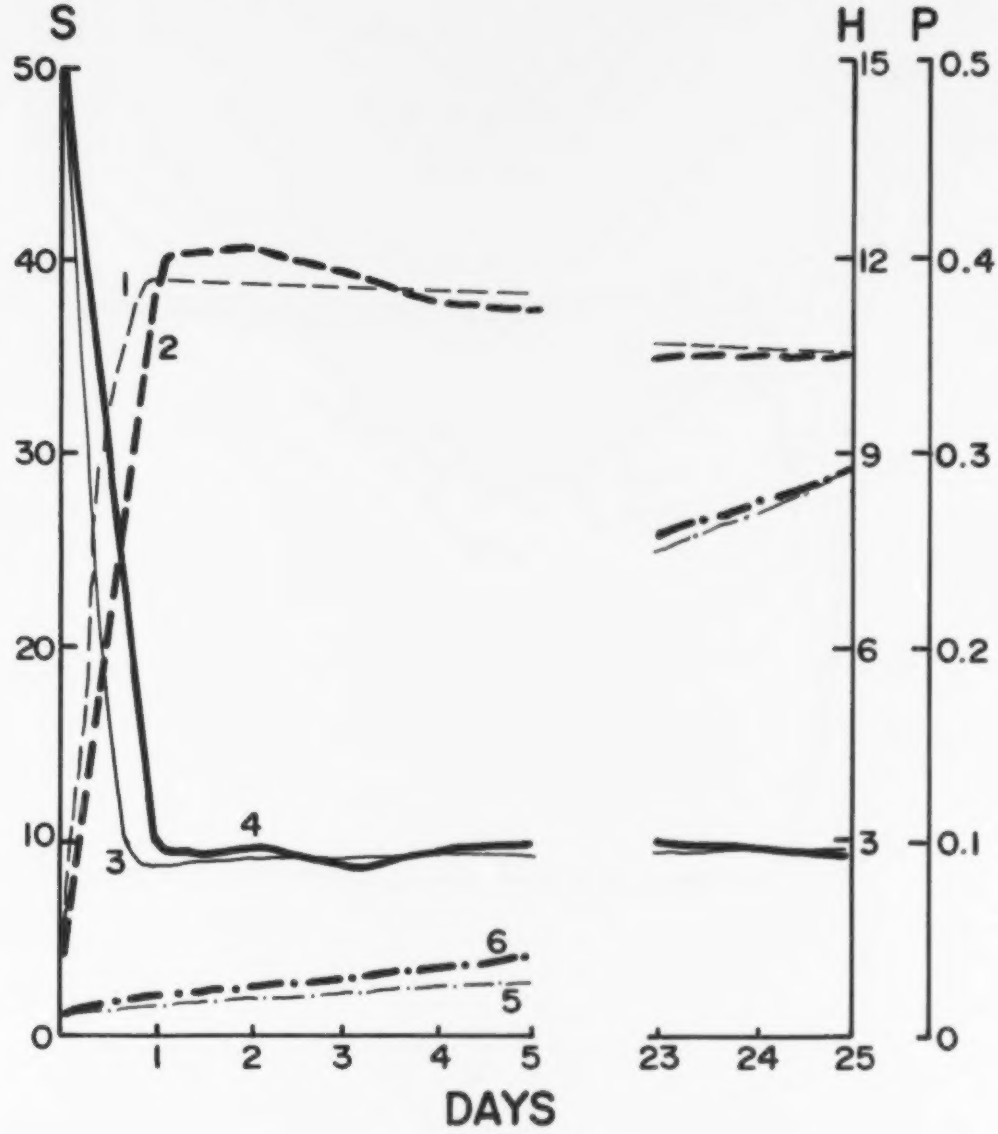

Fig. 10. Change in component values in time -- experiment 10. Symbols are the same as for figure 8.

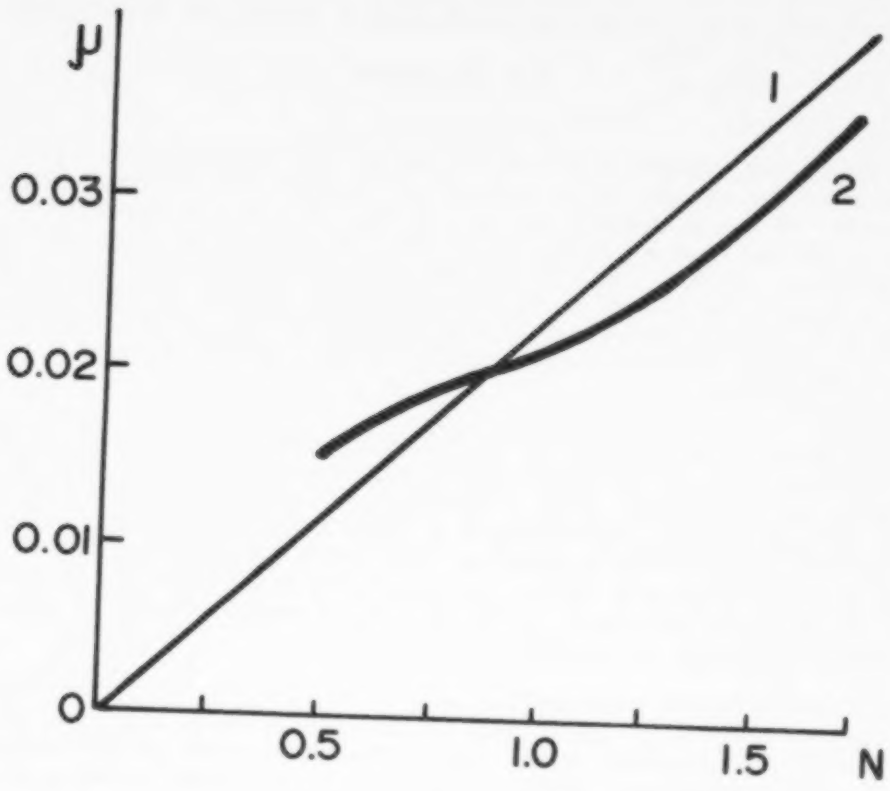

Fig. 11. Dependence of the specific growth rate of bacteria μ on the nitrate nitrogen N concentration. 1 - approximating function $\mu = 0.023$ N2 - experimental curve.

SECTION 14

SIMULATION OF POTENTIAL POLLUTANT-CAUSED CHANGES IN THE ECOSYSTEM, RESULTING FROM THE SENSITIVITY OF AQUATIC ORGANISMS TO TOXICANTS

N.S. Stroganov[1]

The ecosystem changes in a complex manner from exposure to pollutants. The course of the changes which take place depends both on the properties and quantity of the pollutants and on the features of the system itself. Aquatic organisms have a substantial effect on water properties, but their vital activity, in turn, depends on the physical and chemical conditions of the water body. The effect of seasonal climatic changes are superimposed onto these interdependences. Beside the direct effect of pollutants on aquatic organisms, secondary intermediate effects create a "web" of links and interdependences.

It is possible to simulate a complex ecosystem using a small volume, in which there are all of the basic components characteristic of natural aquatic communities. However, such a simulation is expensive, very time-consuming, and requires the equipping of a large experimental base. Another means of simulation could be based on laboratory tests and conducted according to a definite plan using representative organisms from basic functional groups which take part in the cycling of materials and of organisms which are of interest to industry.

Aquatic ecosystems were created over a very lengthy period of time, and have acquired a definite qualitative structure, which is disturbed on a small scale by the seasons of the year. The introduction of pollutants, especially toxic substances, drastically disturbs the established order, and the system passes into a new state, which is in response to the new condition. As a rule, the new state of the ecosystem is unstable. The change which has taken place is not desirable for man, his health, or his industrial activity. The instability is expressed by the disappearance or decrease in numbers of commercially important species of organisms and the deterioration of water quality. Considering the fact that aquatic organisms are a major component of the ecosystem and determine the desirable properties of the water body and the productivity of the species useful to man, we turned our attention first and foremost to them, devoting special attention to the sensitivity of these aquatic organisms to toxic substances--responses which can be determined under laboratory conditions.

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[1] Biological Faculty of Moscow State University

The link between functional groups is expressed diagrammatically in Figure 1 using only the most general aspects that occur in the water body.

The following considerations are relevant to the interrelations in question:

1. The primary organic material (algae, macrophytes) produced is almost entirely transformed into a "new organic material", which is partially utilized by man in the form of commercial animals (fish, crayfish, Mollusca, etc.).

2. Organisms which have died, as well as food residues, are destroyed by bacteria, fungi, and protozoans into simple organic and mineral materials, which enter again into the cycle of materials in the reservoir as nutrients for algae, macrophytes, etc.

3. A portion of the organic material of animal and plant origin does not mineralize and is deposited in the bottom sediment. Man, using a given water body, is interested in high quality water and commercial organisms of good quality. If a given system of interrelationships is in equilibrium, then nearly all of the organic material is transformed and little forms bottom residues. Such a situation exists in oligotrophic reservoirs. The introduction of toxicants into such a reservoir sharply changes the relationship between functional groups because the species have both low and high sensitivity. Some increase in number, others disappear or decrease, and still others remain in their previous state (Figure 2).

In each functional group. there are several dozens of species in unequal numbers. Usually, 2-5 species are dominant in numbers and biomass and the rest are supplementary species thst play a small or negligible role in the interrelationships between aquatic organisms. With a change in the environment as a result of pollution, the relationship between species changes depending on their sensitivity. The resistant species increase and achieve dominance (the development of blue-green algae, inferior fish, etc.). The new relationship of species affects water quality and commercial species. As a rule, this change is less desirable for man's uses.

One can, with sufficient reliability, identify the physiological basis of the change in relationship of species by means of laboratory bioassays of the sensitivity of the principal aquatic organisms which cycle materials in the water body. Based on these data, the weak link in the chain of transformations and interrelationships during exposure to toxic substances can be identified.

Given in Table 1 are data on the sensitivity of various aquatic organisms resulting from bioassays of toxicants. Shown in the table are the permissible, i.e., almost harmless, concentrations of the toxicants in water based on the aquatic organism's sensitivity. The numerical data given in the table reflect both the no-effect and permissible concentrations of the toxicants in the water body at which vital life processes are possible. In accordance with the accepted method (N.S. Stroganov, 1971), at these concen-

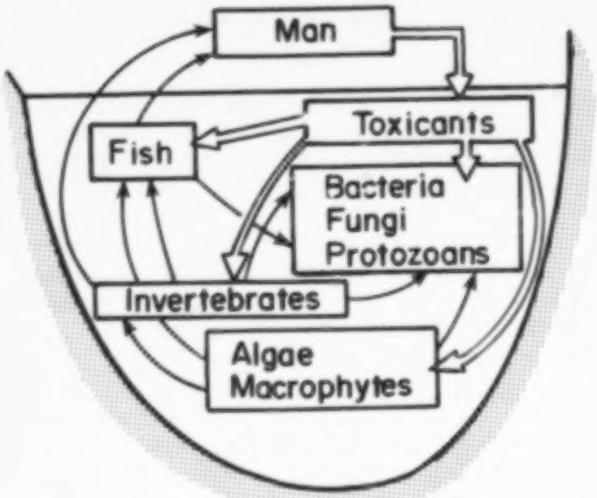
Man
Toxicants
Fish
Bacteria
Fungi
Protozoans
Invertebrates
Algae
Macrophytes

Fig. 1. Block-diagram of the basic functional links in the reservoir

Key: a. Man
b. Fish
c. Toxicants
d. Bacteria
Fungi
Protozoans
e. Invertebrates
f. Algae
Macrophytes

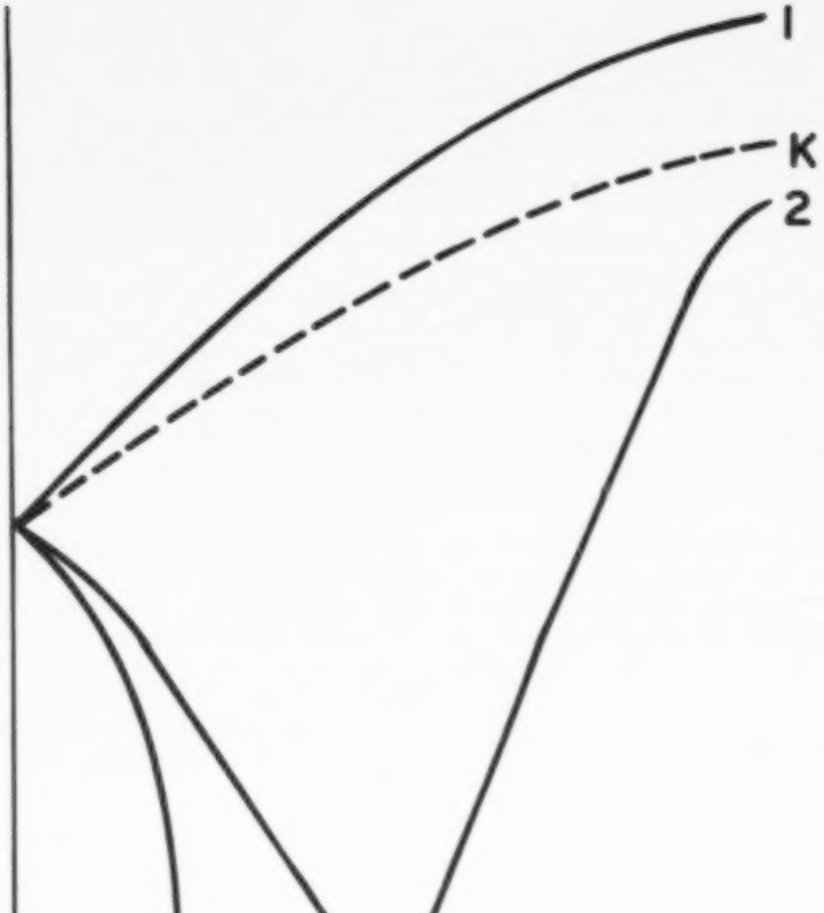

Figure 2. Change in numbers of a species with exposure to a toxic substance. Designations: K--control; 1--low concentration--stimulation; 2--concentration of substance at which 95-97% of individuals dies. The individuals which survive re-establish the population; 3--complete death.

trations effects such as death or abnormalities do not increase by more than 25% as compared with the control. We regard the stimulation of fertility, number of the species, growth rate, and other items as desirable effects of the toxicant. Therefore, such stimulation does not limit the permissible concentration. From this point of view, such concentrations are considered harmless to the test species and the system as a whole. It follows from Table 1 that different aquatic organisms possess different sensitivities to the same toxicant and different manifestations of vital activity are affected in different ways. For example, the processes of reproduction and fertility are disturbed more quickly than mortality. As a result, aquatic organisms which differ in their resistance determine the wide variation of potential changes that occur in the ecosystem.

Variable damage to aquatic organisms from different systematic groups eliminates some species and increases others in the system. An ecological system is unified and all of its components are interdependent. The complicated plant-animal complex not only depends on the abiotic environment, but on the biotic factors (bacteria, protozoan, algae, and higher plant groups) that compose the environment. The diversity of changes in the qualitative and quantitative composition of the organisms is primary a result of the variable sensitivity of the aquatic organisms composing the system.

It is evident from Figure 1 that at a salicylanilide concentration of 1 mg/liter, biological oxidation of organic substances will occur completely but nitrification will occur poorly and incompletely. Consequently, basic self-purification processes will be disturbed and will not be complete. This in turn will create unacceptable conditions for other aquatic organisms. If the salicylanilide concentration were 0.1 mg/liter, then mineralization will be complete and such algae as Scenedesmus, Anabaena and Elodea die, and Ceratophyllum will exist normally. Mollusca, worms, crustaceans, and fish disappear from the community.

In a natural situation, the changes which have been described will occur in a more complex manner because the toxicant will decompose or degrade to another, less toxic state, and because non-resistant individuals will be eliminated. As was evident from Figure 2 (curve 2), a portion of the resistant individuals survive (2-3%), and re-establish the population to normal. Such population responses to the effect of a toxicant have a decisive effect on the structure of the community, and the change in its structure with time. This reaction, as is evident, occurs because individuals have varying sensitivities. Apparent differences in degree of reaction of different aquatic organisms are relative and time-dependent in nature.

By comparing the sensitivity of aquatic organisms of varying systematic groups, one can see that bacteria and algae are less sensitive than fish and Daphnia. However, the magnitude of the difference also depends on the chemical nature of the toxicant. For example, the algae Scenedesmus and Anabaena, are more sensitive to salicylanilide than the bacteria Nitrosomonas and Nitrobacter, while, conversely, the bacteria are more sensitive to 8-oxyquinoline. We tested several dozen substances according to the diagram indicated in Table 1. With rare exception, the bacteria which take part in the decomposition of an organic substance are more resistant to toxicants than invertebrate animals and fish. Algae, as a rule, occupy an intermediate position. Consequently, these empirical data make it possible to construct a diagram to search for the weakest link in the functional cycles of materials in the ecosystem, and provide for a means to forecast changes in the structure of the ecosystem and the relationship of species in the community. Various concentrations of phenylmercuric acetate cause some groups of organisms to drop out. At 0.0005 mg/liter the vital processes of all aquatic organisms will occur normally (see Table 1) and the water can be considered acceptable biologically. If mercury should accumulate in commercially important organisms and damage their commercial quality, then the economic norm would be disturbed, but not the biological norm. In this case, additional analysis should be conducted to limit the concentration of the substance in food organisms. At 0.01 mg/liter the processes of self-purification will occur normally, but some algae and crustaceans such as Daphnia will die and the growth of some fish (trout fry) will be poor. At 0.1 mg/liter the processes of self-purification will be disturbed but the second phase of nitrification occurs. Some algae, macrophytes, worms and crustaceans die, fish feeding is disturbed, and fish fry die. At 1 mg/liter all the organisms indicated in the table die, and only the saprophytic microorganisms which carry out biological oxidation of the organic material remain.

The diagram of sensitivity of aquatic organisms of different systematic groups to a toxicant provides a scientific basis for predicting potential changes in communities exposed to toxicants at different concentrations. By revealing the weak links in the community of aquatic organisms in the food chain, such as through the effect of blue-green algae metabolites, it is possible to foresee the nature of the structural rearrangements of the community.

The means we proposed for simulation of the potential changes in the ecosystem of a reservoir with pollution cannot be without error. Like any model, the proposed simulation has an approximate nature, and the natural situation may be different. But we think that the proposed diagram of simulation is more complete and reliable than those proposed by other researchers to judge the potential changes based on the reaction of one or two organisms, or on biophysical bases. The simulation has two weaknesses.

1. In a natural water, several dozen, and sometimes even hundreds, of species of aquatic organisms co-exist. We conduct tests only on representative species, i.e., predominant ones and species having commercial significance. The rest of the species, a quantitatively larger group but less dominant, does not determine the nature of the community. With a change in conditions, such as the appearance of a toxicant, some dominant species can disappear and less dominant species increase in numbers.

2. We analyzed the toxicity of only one substance, but in natural water under present conditions, several toxicants act simultaneously. Mutual intensification or weakening of their effect is possible. We think

it is correct to sum the toxicity of toxicants. The occurrence of antagonism and synergism is not encountered frequently, and can be disregarded. It is difficult to predict the hydrochemical situation into which the toxicant enter. Its effect on the aquatic organisms will depend on temperature, dissolved O_2, pH, total hardness and other indicators. Prediction is facilitated by the inclusion of changes in the toxicity of toxicants (metals, pesticides, etc.) caused by a series of environmental factors. Thus, for example, a reduction in temperature usually lowers the toxicity of the substance; a decrease in the environment's pH increases the toxicity of metals; and an increase in the water's hardness leads to a reduction in toxicity. One can also list other factors which have an effect on the final toxicity. We should not be discouraged by the difficulties in applying laboratory tests to forecast changes in the ecosytem when exposed to toxicants. For specific water bodies perhaps it will be necessary to change the experimental organisms. For examination of aquatic ecosystems, considerable difficulty arises in finding major components which determine its behavior. Simplifying the approach to solving the problem, I selected basic functional blocks in the cycle of materials and the energy flow in the system. They were known long ago, and have not been disputed. However, we know little about the links within the blocks, and they often appear as "black boxes", about which we judge based on what comes in and goes out. For better predictions it would be desirable to know more.

The matter of the stability of the system and the mechanisms of its regulation arises in connection with predicting potential changes in the ecosystem. It seemed correct to me to single out the two major aspects in this matter--the abiotic environment and the organisms. The stability of the ecosystem is determine primarily by the stability of the environment. The introduction of toxicants (pollutants) changes the aquatic organism's living environment and, as a result, a change in the aquatic community occurs. Specific diversity is primarily determined, apart from the historically established conditions, by hydrochemical and hydrological conditions. However, the organisms of the functional groups can maintain the stability of the system within known limits. This is demonstrated especially well when some species increased their numbers or when a new species appears. But the abiotic environment always plays the largest role in the stability of the ecosystem. Changes in this environment often have a decisive effect on the nature of the links in the system. The different relationships of aquatic organisms to the environment, which contains toxicants, is the basic relationship.

TABLE 1. COMPARISON OF HARMLESS CONCENTRATIONS OF SUBSTANCES (MG/LITER)

Organisms	Indicators	Toxicants			
		1	2	3	4
	BOD[1]	50	20	50	5
Bacteria	NO formation	0.5	10	0.01	0.1
	NO formation	0.5	15	0.01	0.01

Organisms	Indicators	Toxicants			
		1	2	3	4
Algae:					
Chlorella vulgaris	Number of cells	-	-	1	0.1
Scenedesmus quadricauda	Number of cells	0.001	0.01	-	-
Anabaena variabilis	Number of cells	0.01	0.1	10	0.005
Macrophytes:					
Ceratophyllum demersum	Growth of stalk	1	3	-	0.05
Elodea canadensis	Growth of stalk	0.01	-	-	0.5
Lemna minor	Growth of roots	0.1	-	-	0.01
Mollusca:					
Limnaea stagnalis	Survival rate of adults	0.1	0.5	-	-
Planorbis planorbis	Number of eggs laid	0.7	-	0.5	0.1
Planorbis planorbis	Hatching	0.02	0.005	-	0.2
Planorbis planorbis	Survival rate of young	0.02	0.01	1	-
Worms:					
Tubifex tubifex	Survival rate	0.02	0.01	0.005	0.05
Crustaceans:					
Daphnia magna	Survival rate	0.005	0.001	0.001	0.001
Daphnia magna	Reproduction of adults of initial individuals	10	-	1	-
Daphnia magna	Number of generations	0.01	0.001	0.01	-
Daphnia magna	Number of offspring	0.005	0.0005	0.001	0.0005
Daphnia magna	Molting	10	0.01	1	0.02
Insects:					
Chironomus plumosus larvae	Survival rate	0.005	0.005	0.01	0.1
C. plumosus larvae	Pupation	0.005	0.01	0.01	-
C. plumosus larvae	Emergence	0.005	0.01	0.01	-
Fish:					
Rutilus rutilus	Embryonic development	0.005	5	-	-
Rutilus rutilus	Hatching of fry	0.005	5	-	-
Rutilus rutilus	Survival rate of fry	0.005	0.1	-	-

Organisms	Indicators	Toxicants			
		1	2	3	4
Salmo gairdneri iridens (yearlings)	Feeding	0.1	0.1	0.005	0.01
S. gairdneri iridens (yearlings)	Growth	0.005	0.1	0.005	0.002

Toxicants: 1--salicylanilide; 2--sodium pentachlorophenolate;
3--8-oxyquinoline; 4--phenylmercuric acetate

Temperature 18-22. Length of test 30 days (10-20 days for the bacteria). Survival rate is indicated for 50% of individuals.

[1] Biochemical Oxygen Demand

SECTION 15

FISH-POPULATION STUDIES IN THE OHIO RIVER

William C. Klein

INTRODUCTION

In 1957 the Ohio River Valley Water Sanitation Commission (ORSANCO) and the Kentucky Division of Fish and Wildlife initiated a 3-year study - Aquatic Life Resources in the Ohio River (ALRP). Chemical fishing in navigation locks of the U.S. Corps of Engineers was used as one of the principal methods for collecting the samples to be analyzed. Subsequently, from 1968 to 1970, state, federal, and inter-state agencies continued the investigations by cooperative arrangement and gathered data to establish a relationship between trends in the fish population and changes in water quality occurring from the installation of improved wastewater treatment facilities. The goal of these ongoing studies was to provide agencies responsible for water quality management in the Ohio River region with information necessary for assessing river quality conditions.

In 1974 ORSANCO began to expand its monitoring program on the Ohio River and the lower reaches of its major tributaries to contribute needed information to these agencies. An important part of the program, biological monitoring at selected locations, was partially initiated during the fall of 1975; again chemical fishing was used. Some background together with a summary of the methods used and the results obtained from the previous studies, is detailed below.

THE OHIO RIVER

The Ohio River is a large canalized stream extending 981 miles from Pittsburg, Pennsylvania, where it is formed by the Allegheny and Monogahela Rivers, to Cairo, Illinois, where it flows into the Mississippi River. At normal pool stages the stream varies in width from approximately 1,000 ft to 4,000 ft in the lower reaches. Flow patterns in the river are extremely variable, ranging from 6,600 cu ft/sec[1] in the upper river to 48,5000 cu ft/sec[1] in the lower reaches. Presently, its depth is controlled by a series of high- and low-level dams and associated navigation locks at some 21 locations. The U.S. Corps of Engineers maintains a minimum 9-ft channel for navigation purposes. The Ohio River receives the flow from 19 major tributaries and over 100 reservoirs, in addition to discharges from about 295 municipalities and 200 industries. Although the flow in the Ohio River is quite variable, it is highly regulated by the U.S. Corps of Engineers through their manipulation of the reservoir and dam system (see map).

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[1] Minimum 7-day in 10-year flow.

The change in river regime caused by its canalization and increased pollution load has altered the species composition and relative abundance of biological organisms from those found and recorded by the early white settlers, and changes in dam construction have had an impact upon migratory patterns of fish. The old wicket dams, for instance, permitted open-river conditions for many months of the year, and fish were free to travel from one pool to another. The installation of the fixed structure high-level dams across the river does allow fish to travel from one pool to another, although this does not occur in the same free manner as before. In addition, the change in variety of food organisms has been substantial and was probably more influential ecologically than any changes in water quality. These alterations must be taken into account when evaluating the results of the lock-chamber studies. For example, the shift from a free-flowing stream with a significant slope to a canalized river separated by a series of low-level locks and dams and then to the present condition of longer, deeper pools separated by fixed structured dams has significantly modified biological habitats to the extent that many previously abundant species such as the sturgeon and the paddle-fish are now reduced in number and their distribution limited. Some species of fish (such as the deep-bodied suckers, the gizzard shad, and perhaps some of the smaller sunfish) have increased in abundance, for the lakes created by the dams favor them. Carp, another species introduced into the Ohio, shows strong preference for the quiet waters furnished by the dams.

From the standpoint of spawning and reproduction of fish, the Ohio exhibits many of the traits of a large canalized river. The stream is characterized by a gravelly bottom, a paucity of shallow water and very few, if any, riffles or weeds suitable for nesting. Shore lines in many localities show the effects of bank erosion caused by the large variations in river flow and to a lesser degree the backwash of commercial and recreational boats navigating the river. As a result, the number of areas suitable for spawning takes place in small creeks and tributaries. Sampling performed during the course of the ALRP, however, revealed that a large number of species requiring shallow water, weeds, and riffles to reproduce were in fact spawning in the small tributaries and then returning to the main stem. The sauger, round-bodied suckers (red horses), largemouth and smallmouth bass, and golden shiners prefer the small tributaries with shallow gravel bars and weeds for spawning, but they are found throughout the Ohio as both fingerlings and mature fish.

CHEMICAL FISHING

Various chemicals (rotenone, toxaphene, creosol, copper sulfate, and sodium cyanide) have been used in fish sampling. The most acceptable of these has been rotenone because of its high degradability, freedom from such problems as precipitation and persistent toxicity, and above all, the relative safety for the user. Rotenone, which is obtained from the derris root (Deguelia elliptica, East Indies) and the cube root (Lonchocarpus nicour, South America) has been used extensively since 1934 in fishery work throughout the United States and Canada. The chemical is toxic to man and warm-blooded animals (132 mg/kg), but has not been considered hazardous in the concentrations used for fish eradication (0.025 - 0.050 mg/liter active ingredient). Therefore, it has been employed in waters used for bathing and in some instances in drinking water supplies. Activated carbon removes rotenone very effectively, as well as the solvents, odors, and emulsifiers present in almost all commercial rotenone formulations. The rotenone used in lock-chamber studies is a 5% active ingredient in an emulsion base. Best results are obtained with water temperatures above 13 C (55 F). It is a relatively fast-acting toxicant which decomposes in 24 hr or less. The toxicity threshold, however, differs only slightly among fish species, and for this reason rotenone cannot be used as a selective toxicant for certain species.

LOCK-CHAMBER APPLICATION

The lower gate of the lock chamber is left open approximately 4-6 hr before the sampling. Personnel move into the lock chamber in boats, and the lower gates of the chamber are closed. The rotenone emulsion is then pumped and sprayed from a boat into the water within the lock chamber until a concentration of 1 mg/liter is attained. The chemical is then rapidly dispersed through the water inside the chamber by means of the ouboard motors on the boats. The fish begin rising to the surface 5-10 min after the rotenone has become well mixed. As the fish surface, personnel in boats pick them up with dip nets and place them in large tubs. Because of the size of the chambers, i.e., 100 ft by 1,000 ft, approximately five boats and 10 men are required to conduct the work. Additional personnel spot the fish as they surface. After all the fish have been picked up and placed in receptacles, the lock-chamber gate is partially opened, and the water is permitted to bleed out slowly. The fish are then taken to a central location near the lock and are sorted, weighed, identified, and catalogued. Appropriate species, such as the channel catfish, are frozen in dry ice, shipped to laboratories, and analyzed for heavy metals and pesticides. Recently, the U.S. Food and Drug Administration has cooperated in analyzing the fish for these constitutents. The fish are also inspected for parasites and other pathological indications possibly attributable to adverse water quality conditions. Fish not used in the additional studies are disposed of by burying.

SAMPLING RESULTS

A comparison of lock-chamber sampling results for the 1957-60 and 1968-70 periods reveals that a number of changes have taken place in the composition of the fish population in the Ohio River, reflecting alterations in physical, chemical, and hydrological conditions such as the replacement of old style wicket dams, increased water pollution control, and augmented stream flow from reservoirs during the low-flow period. The changes appear to parallel and substantiate physical and chemical water quality trends noted during the same period.

In the 1957-60 period the 10 most abundant species of fish in the population samples were in descending order: the emerald shiner, gizzard shad, freshwater drum, mimic shiner, channel catfish, silver chub, black bullhead, threadfin shad, blue catfish, and sand shiner. The 10 species that contributed the greatest total weight in the samples were gizzard shad, carp, channel catfish, freshwater drum, emerald shiner, skipjack herring, flathead catfish, blue catfish, black bullhead, and river carpsucker.

In comparison, the 1968-70 sampling revealed that six of the 10 most abundant species in the population--the gizzard shad, emerald shiner, freshwater drum, channel catfish, bullhead, and the drum--were retained from the 1957-60 sampling period. The remaining species were replaced by the carp, black crappie, yellow bullhead, and river carpsucker. Of the 10 species that contributed the greatest total weight in 1957-60 the gizzard shad, carp, channel catfish, freshwater drum, river carpsucker, flathead catfish, and black bullhead continued in that category. The three new species were bigmouth buffalo, white crappie, and bluegill.

The species composition also varied throughout the river as it did in 1957-60. In the upper third the most abundant species were carp, channel catfish, gizzard shad, emerald shiner, and brown bullhead; in the middle third, the carp, gizzard shad, channel catfish, mimic shiner, and skipjack herring; and in the lower third, the gizzard shad, channel catfish, emerald shiner, freshwater drum, and bigmouth buffalo.

Among the changes noted during the 10-year period was the marked increase in the carp population, which had not been so predominant in the earlier studies, and the increased abundance of species sought by both sport and commercial fishermen - largemouth and smallmouth bass, sauger, crappies, and sunfish. It is believed that the new deeper pools with decreased velocities and more lake-like settings probably account in large measure for the increased carp population. The re-emergence of significant numbers of the so-called sport and commercial species is probably due to the decreased water pollution loads going to the river. Such a conclusion is supported by ORSANCO appraisals of water quality conditions. Of the 21 water quality characteristics routinely monitored by ORSANCO, all except four are now meeting established criterial goals for streams in the compact area.

SUMMARY

A review of historical and recent information concerning fish in the Ohio River during 1957-60 and 1968-70 indicates that the composition of the fish population has changed during the period. In large measure, the

changes can be attributed to the canalization of the river and increased pollution load. Although the pollution load has been decreased in recent years by the installation and operation of wastewater control facilities, the lake-like setting of the Ohio River continues to influence the kinds and numbers of fish in the river, as evidenced by the chemical fishing studies performed in the lock chambers. Although many of the so-called sport and commercial species have returned to the river, the fish species desiring a lake-like setting continue to dominate the population.

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SECTION 16

REGISTRATION OF PESTICIDES: CONSIDERATIONS IN CONDUCTING AQUATIC TOXICITY TESTS

Richard A. Schoettger

Reports of the U.S. Tariff Commission show that production of synthetic organic chemicals amounts to billions of pounds per year. Pesticides alone account for more than 1 billion pounds, of which about half are insecticides and the remainder are herbicides, fungicides, and other control chemicals (Fowler and Mahan, 1973). Extensive use of persistent pesticides for over a quarter-century, often without due concern for direct and indirect contamination of fish and wildlife habitats, has resulted in our unwitting use of these resources as biologic indicators of contamination (Johnson, 1968; Henderson, Johnson, and Inglis, 1969; Katz et al., 1970, 1971, 1972; Day, 1973; McKim et al., 1973, 1974). These fish and wildlife resources are valued at more than $7 billion per year (U.S. Fish and Wildlife Service, 1972). The lethal effects of pesticide spills, careless applications, and point and non-point discharges have been relatively obvious for years, but not the subliminal effect of sublethal concentrations. Recent improvements in analytical technology and nationwide sampling by national monitoring activities have revealed a broad array of pesticide and industrial chemical residues in various kinds of fish and wildlife and their habitat. These findings show that trace quantities of pesticides can be mobile and accumulative in aquatic ecosystems. With new, multidisciplinary research approaches, scientists are now beginning to demonstrate what they suspected for years--that sublethal concentrations of pesticides and other contaminants may have subtle and adverse effects on basic life and behavioral processes of fish and wildlife. The scope of these processes determines an organism's ability to cope with continuous competition and natural stresses. Chemical contaminants are added stresses to which fish may or may not be able to adjust, and populations may be subtly modified or attenuated. Therefore the U.S. Fish and Wildlife Service must anticipate to the best of its ability, through its own research and in cooperation with other agencies and institutions, the ecological implications of known, suspected, or potential chemical contaminants.

In view of documented effects of pesticides on fish and other aquatic life and the apparently ubiquitous distribution of certain pesticide residues in aquatic habitats, it seems reasonable to assume that past research requirements for pesticides have not been adequate to anticipate effects on these resources. In 1970-71 the U.S. Fish and Wildlife Service reorganized and integrated scientific disciplines at the Fish-Pesticide Research Laboratory (Figure 1) so as to develop anticipatory rather than documentary information concerning effects of pesticides on fish and fish-food organisms (Grant and Schoettger, 1972).

High priority at the laboratory is given to research in four topical areas:

1) Agents developed for use in fishery habitats (control of aquatic weeds, algae, slime, mosquitos, mollusks, and fish);

2) agents intended for use on land and water adjacent to or contiguous with fishery habitats (forest insect control, ditch bank management, forest fire retardants);

3) agents manufactured in large volume and used widely (selected agricultural and industrial chemicals); and

4) known contaminants of wild and propagated fish and their food and habitat.

The investigational divisions outlined in Figure 1 are intended to reflect the kinds of studies that should be considered in the development and registration of pesticides. The principal systems are ordered from relatively routine and short-term studies to more complex and lengthy investigations. All or parts of the framework may be used depending on the extent and applicability of biological and chemical data already available, intended use pattern(s), and target pest(s).

The framework is designed around fish as the primary test animal, but it is also compatible with parallel investigations essential to anticipating pesticide effects on fish-food organisms. In all studies the investigator must include sufficient test animals and replications to estimate statistical significance of results. Sources, general physical conditions, disease treatments, and holding conditions (such as photoperiod, diet and feeding rate, water characteristics) of test animals should be reported. Whenever possible, test animals, diets, and holding waters should be chemically analyzed to document pre-exposure of test animals to pesticides or other contaminants. Analytical chemistry reports should document results for reagent blanks, limits of sensitivity and detection, reproducibility, recovery efficiency for extracts, and sample variability.

The investigational sections within the research framework are divided into principal systems and support systems; consequently, researchers in two or more research divisions generally integrate their efforts to achieve common goals. Typical investigations generated by this framework include some 11 types of studies:

(1) Acute toxicity, and variations among species and water types;

(2) teratogenicity;

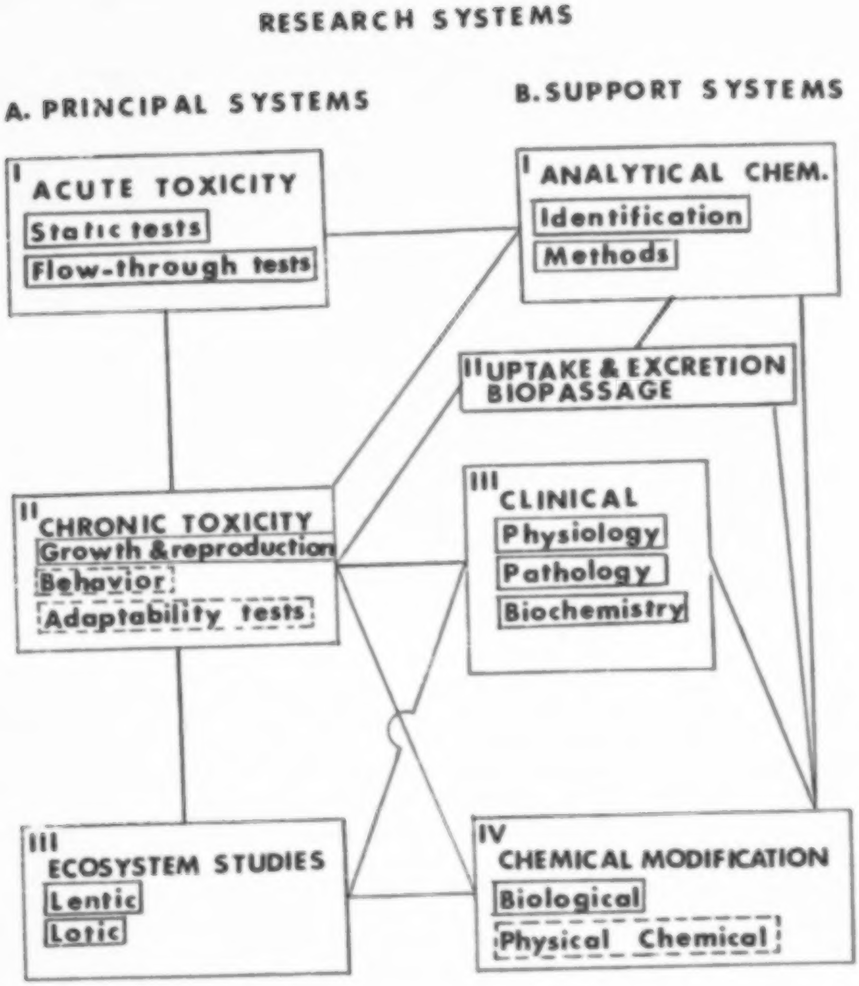
RESEARCH SYSTEMS
A. PRINCIPAL SYSTEMS
B. SUPPORT SYSTEMS
I ACUTE TOXICITY
Static tests
Flow-through tests
II CHRONIC TOXICITY
Growth & reproduction
Behavior
Adaptability tests
III ECOSYSTEM STUDIES
Lentic
Lotic
I ANALYTICAL CHEM.
Identification
Methods
II UPTAKE & EXCRETION BIOPASSAGE
III CLINICAL
Physiology
Pathology
Biochemistry
IV CHEMICAL MODIFICATION
Biological
Physical Chemical

Figure 1. Organization of investigational divisions at the Fish-Pesticide Research Laboratory, Columbia, Mo.

(3) biological uptake, storage, and elimination;

(4) sublethal effects on growth, reproduction, and morphogenesis;

(5) physiologic-biochemical effects and the organisms' homeostatic ability to adapt to natural stresses;

(6) effects on energy transfer and physical, chemical, and biological interrelations in aquatic ecosystems;

(7) methods for identifying and quantifying pesticides, their degradation products, and other contaminants;

(8) physiochemical factors affecting molecular structure and biologic activity;

(9) effects on behavior;

(10) methods for eliminating or deactivating chemical residues; and

(11) correlations of residues with their biologic activity.

Mount (1967) pointed out that numerous past studies on toxicological and physiological effects of pesticides in fish have yielded few data that can be used to correlate these effects and chemical-residue measurements with significant damage to aquatic forms. Therefore, investigators must keep in mind the potential interpretive value of anticipatory research on pesticides. In-depth experiments should be designed so that they demonstrate effects of pesticides on aquatic organisms, but they should also include measurements of residues induced by test concentrations which are commensurate with concentrations recommended for use of the pesticide. Such data are essential to experimental designs for field evaluations of pesticides and for interpreting significance of unintentional contamination of aquatic ecosystems.

The research framework discussed above, along with a more detailed account of guidelines for conducting toxicological research with aquatic organisms, was published by the National Academy of Sciences in 1973.

REGISTRATION REQUIREMENTS

For the most part, pesticides must be registered in the United States according to provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A number of provisions in this act were most recently amended by the Federal Environmental Pesticide Control Act of 1972. Responsibility for implementing FIFRA, as amended, is vested in the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). In general, properties of pesticides that must be researched in the registration process include (1) efficacy on the target pest, (2) general and environmental chemistry of the pesticide, (3) safety to the applicator and the consumer of treated products, and (4) effects on non-target organisms, including those of aquatic ecosystems. More specifically with regard to aquatic organisms, the administrator (EPA) "shall register a pesticide product or approve amended and supplemental registration if he determines that, when considered with any restrictions imposed, the pesticide will perform its intended function without unreasonable adverse effects on the environment" (Environmental Protection Agency, 1975a).

If a pesticide is intended for outdoor use, or for use where it may contaminate water, applicants for registration must submit data that will permit evaluation of hazards to non-target animals, as given in the proposed registration guidelines (Environmental Protection Agency, 1975a). The minimum requirements include the acute toxicity (96-hr LC50) of the technical grade pesticide for both a coldwater and warmwater species of fish, such as rainbow trout (Salmo gairdneri) and bluegills (Lepomis macrochirus). An acute test must also be performed on an aquatic invertebrate, such as a daphnid. Data reports must include calculations of the dose-response line, the 95% confidence intervals for the LC50, and the slope of the regression. Other studies may be required, depending on whether acceptable research demonstrates that, under conditions of proposed use, the pesticide causes no unreasonable adverse effects on plants and animals of aquatic ecosystems. Such additional studies, considered "conditional tests," may be judged necessary depending on other factors, such as (1) chemical and physical properties of the pesticide, (2) amount of pesticide applied per unit of area or time, (3) likely degree of contamination in various environmental components according to proposed use, (4) various species to be affected, (5) likely routes of exposure, (6) persistence of the pesticide or its biologically active degradation products and transfer between environmental components, and (7) degree of biological uptake of the pesticide or its significant degradation products.

The "conditional tests" that concern water contamination may include (1) additional acute toxicity tests with the technical grade or formulated pesticide against freshwater and estuarine or marine fish and invertebrates; (2) toxicity-residue studies with bottom-feeding fish (channel catfish, Ictalurus punctatus, or carp, Cyprinus carpio), predaceous fish (e.g., largemouth bass, Micropterus salmoides, bluegills, or trout), molluscs (oysters or freshwater clams), crustacea (Daphnia sp., Gammarus sp., or crayfish), and insect larvae; (3) studies of chronic effects on reproduction of fish or invertebrates or both; and (4) "special studies" (actual or simulated field studies in which proposed use patterns are tested). Other toxicity data may be required where unusual or specific potential hazards may be associated with a particular proposed pesticide use. At present, when a pesticide is proposed for aquatic use, the applicant for registration is required to provide data to establish a residue tolerance in edible tissues of fish, shellfish, or both, or obtain an exemption from the requirement for a tolerance. Research guidelines for establishing tolerances have not yet been proposed. In general, however, such research would probably include oncogenic evaluations; chronic feeding studies in animals for the measurement of effects on the central nervous system and the hematopoietic system; and histological changes in the liver, kidney, and male and female reproductive systems.

Registration or re-registration of a pesticide may be questioned if the proposed use could result in an average concentration, in water 6 inches deep, greater than 0.5 of the LC50 for representative aquatic organisms. Pesticides giving an average concentration in 6-inch-deep water of between 0.1 and 0.5 of the LC50 would most likely be classified for restricted use by certified applicators. These criteria also apply to metabolites or degradation products of the pesticide. In addition, a determination of unreasonable adverse effects on the environment must include an analysis of the chronic effects of exposures to pesticides.

REGISTRATION GUIDELINES

The various test methods by which pesticide registration requirements can be satisfied are included as an appendix to "Guidelines for Registering Pesticides in United States" (Environmental Protection Agency, 1975b). In general, methods are organized along the lines of the pesticide registration requirements - i.e., methods are given for the assessment of (1) pesticide efficacy, (2) environmental chemistry, and (3) hazards to humans, domestic animals, fish, and wildlife. The presentation of methods is not intended to imply that they are necessarily standard, inflexible, or the only methods that can be used. However, they are now considered acceptable for developing data to support registration and for planning research and are an excellent source of information. Literature citations are given only for references that are readily available and that describe methods that are acceptable as presented. Modifications of methods are presented as annotated bibliographic citations, and unpublished methods are included as full tests. Applicants for pesticide registrations are encouraged to discuss with EPA the research methods they intend to use.

The aquatic toxicology methods include acute toxicity testing with various marine and freshwater fish and invertebrates, chronic (complete life cycle) and partial chronic studies (includes reproductive phase of life cycle), accumulation tests, and field appraisal studies. The methods classed as "routine" have been used by numerous investigators for many years to investigate a wide variety of toxicants. Those methods classed as "tentative" have been used by two or more toxicologists for several years, but there is no consensus concerning detailed application of the methods, and there are no interlaboratory test comparisons to show consistency of results. "Developmental" methods are those used or proposed by one or a few investigators, and the techniques involved may not be well known and may require that investigators have considerable experience to achieve consistent results.

CHRONIC TESTS

Except for chronic toxicity tests with fathead minnows (Pimephales promelas), chronic or partial chronic tests with various aquatic forms--sheepshead minnows (Cyprinodon variegatus), brook trout (Salvelinus fontinalis), bluegills, daphnids, and midges--are considered tentative or developmental. Because such studies may be required to appraise the hazard of a pesticide to non-target animals, I would like to summarize the results of two studies that we (Staff, Fish-Pesticide Research Laboratory) recently conducted one with toxaphene (Mayer, Mehrle, and Dwyer, in press; Mehrle and Mayer, in press) and one with 3-trifluoromethyl-4-nitrophenol (TFM) (Foster Mayer, personal communication).

Toxaphene Investigations

Growth and reproductive effects--Although use organochlorine insecticides has been reduced in recent years, some including toxaphene are still used extensively. Between 30 and 40 million pounds of toxaphene are currently being applied annually on crops and livestock in the United States. Since use of DDT was curtailed, toxaphene has often been used to replace it, alone or in combination with other insecticides. We therefore began cooperative studies in 1972 with EPA to evaluate the effects of toxaphene on fishery resources.

Toxaphene is acutely toxic to fish; lethal threshold concentrations for brook trout, bluegills, fathead minnows, and channel catfish range from 0.5 to 15.2 µg/liter. In earlier studies we found that growth of adult brook trout was reduced during continuous exposure to 0.29 and 0.50 µg/liter toxaphene, and the added stress of natural spawning caused extensive deaths of adults at these concentrations. Growth and survival of fry were affected adversely at concentrations as low as 0.039 µg/liter, and they accumulated toxaphene residues 5,000 - 21,000 times the water concentration.

Because toxaphene is used extensively on cotton in the southeastern United States, we also tested it against the fathead minnow, an important forage and bait species, and against channel catfish. Ten-day-old fathead minnow fry were exposed continuously to concentrations of 0.06 - 1.2 µg/liter of toxaphene. The fish were reared at a constant temperature of 24 C and under a regulated photoperiod approaching natural lighting. Growth of the fish was not affected in exposures as long as 90 days. Between 90 and 150 days, however, the growth of all fish exposed to toxaphene was significantly less ($P<0.05$) than that of the control fish. At this time toxaphene residues accumulated during this period exceeded 90,000 times those in the treated water. Residues in fish exposed to the highest concentration, 1.2 µg/liter, averaged 94 µg/g.

Two-year-old channel catfish were also exposed continuously to concentrations of 0.023 - 0.51 µg/liter of toxaphene for 4.5 months before spawning (Figure 2). Spawning occurred naturally through manipulation of photoperiod and water temperature, and 85% of the fish that reached sexual maturity spawned. Although the adults were not affected, hatchability of

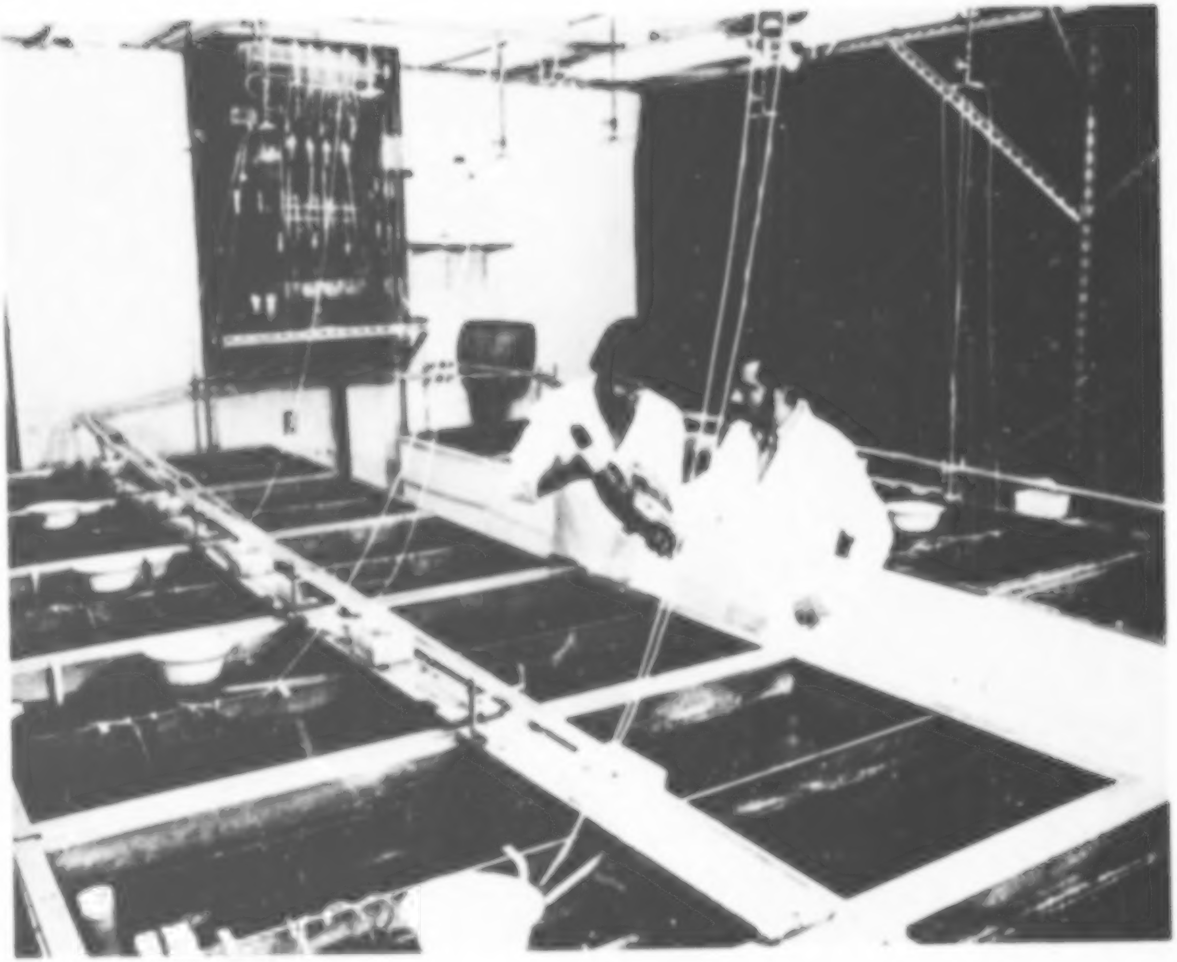

Figure 2. Multiple concentration, flow-through diluter with controlled light and temperature used to determine sublethal effects of toxaphene on growth and reproduction of channel catfish.

eggs from adults exposed to 0.51 μg/liter was reduced slightly, and 0.22 and 0.51 μg/liter of toxaphene increased the mortality of fry and decreased their growth. Toxaphene residues in fry from these two exposures were 8 and 32 μg/g, respectively.

Toxaphene may also have an adverse effect on important natural fish foods. Concentrations of 10 μg/liter or greater inhibited emergence of midge larvae, but this is well above the concentrations affecting reproduction or growth in fish. However, greater resistance of midge larvae could enable them to accumulate significant residues. Reproduction of daphnids was halved when the organisms were exposed to 0.12 μg/liter toxaphene for 21 days, and the no-effect concentration was only 0.007 μg/liter.

Toxaphene concentrations of 0.04 - 0.25 μg/liter are detrimental to the production of fish and their food; consequently contamination of waters supporting these resources by run-off, leaching, or spraying should be avoided. Unfortunately, these low concentrations are very difficult to detect analytically. However, tissue residues exceeding 0.4 - 1.8 μg/g in salmonids may be associated with reduced growth and reproductive success, and residues over 5 μg/g may cause reduction in growth of channel catfish fry.

Biochemical effects--Collagen is the major fibrous protein of all vertebrates and serves as the major component in the organic matrix of connective tissue and bone. The proper ratio of collagen and minerals is essential for rigidity and flexibility of bone, as well as overall development and maturation.

Eggs and fry of brook trout and young fathead minnows were exposed to toxaphene for 90 and 1500 days, respectively, at the same concentrations (0.039 - 0.5 μg/liter and 0.06 - 1.2 μg/liter) as those tested in the growth and reproduction studies reported above. Analyses of backbones showed that synthesis of hydroxyproline, the major amino acid of collagen, was inhibited during the first few weeks of exposure to toxaphene at concentrations of 0.039 μg/liter or higher. In older fish collagen synthesis was reduced at all concentrations of toxaphene by the end of the exposure period and appeared to be correlated with reduced growth. The earliest inhibition of collagen synthesis occurred at the highest toxaphene concentrations and preceded observable reductions in growth.

In general, the net effect of toxaphene in fish was lower collagen synthesis and greater mineralization of the backbone and whole body. We postulated that this condition may cause the backbone of fish to be brittle and fragile and therefore subject to breakage during times of swimming stress. We subjected groups of toxaphene-treated and control fathead minnows to a sublethal electrical shock (60, AC) and then examined the backbones by x-ray. The observations (Figure 3) confirm that the backbones of toxaphene-treated fish seem more fragile and could break while the fish are migrating or escaping predators.

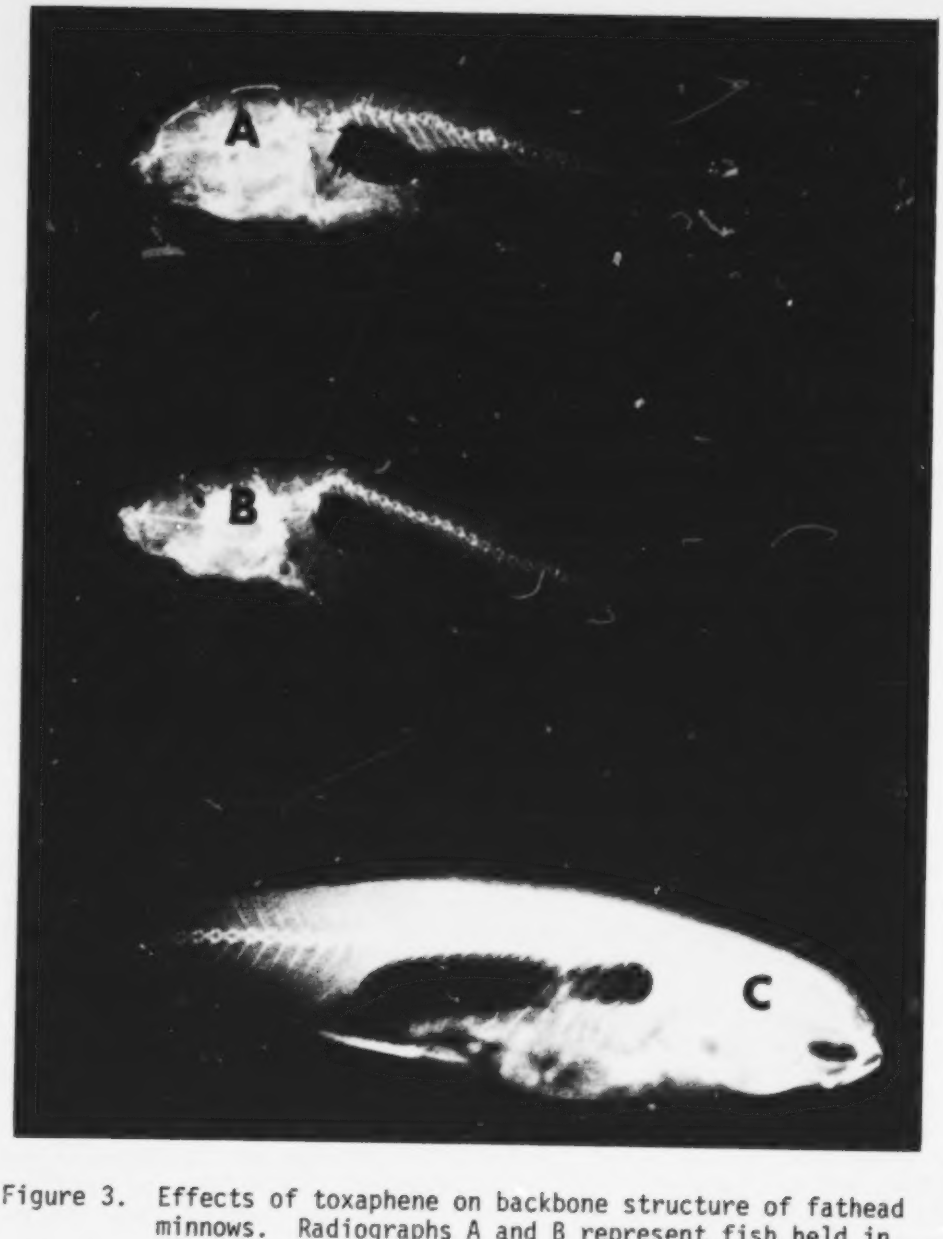

Figure 3. Effects of toxaphene on backbone structure of fathead minnows. Radiographs A and B represent fish held in water with a low toxaphene concentration (0.055 µg/liter); C represents the control group. Arrows show areas of backbone affected.

A condition known as "broken-back syndrome" has been reported by other investigators in pond-reared channel catfish, as well as in natural populations. Studies with fingerling channel catfish showed that exposures for 90 days to concentrations ranging between 0.044 and 0.535 μg/liter significantly decreased collagen and increased calcium in their backbones. Residues in the affected fingerlings ranged upward from 3 μg/g. X-ray analyses of these fish revealed aberrations in backbone structure (Figure 4). Studies to determine the mechanism of action as well as the possibility that other contaminants could also induce this condition are now in progress.

TFM Investigations

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) was registered in 1964 for control of larval sea lampreys (*Petromyzon marinus*) in selected tributaries of the Great Lakes. The EPA is presently renewing registration of TFM on a year-to-year basis while research is being conducted on potential adverse effects and residues in non-target species. Brook trout are an important and indigenous sport fish in many of the streams treated with TFM.

Chronic exposures of adult brook trout to concentrations ranging from 0.7 to 14 mg/liter of TFM formulation (35.7% active ingredient) were begun in 1973. The adults were exposed for 120 days before spawning, and their offspring for 90 days. Growth of adults exposed to the highest concentration was reduced, and all died during spawning. Many of the adults exposed to 14 mg/liter and a few of those exposed to 8 mg/liter developed blindness. Concentrations of 3.3 mg/liter or higher reduced egg viability (as measured by the percentage reaching the neutal keel stage) and hatchability and growth rates of fry.

Although TFM has a significant chronic effect on brook trout at concentrations well below those used to control lamprey larvae, it is not likely that use patterns for TFM would result in such long and continuous exposures. Therefore, we repeated the study, but exposed two groups of adult trout in a light- and temperature-controlled flow-through diluter, in simulation of a typical stream treatment. Because such treatments generally take place during the summer or early fall, one group of fish was exposed to TFM during the summer at 15 C and the second during the fall at 9 C. Both groups were exposed to 16-18 mg/liter of TFM for 12 hr. About 19% of the adults in the first group died shortly after exposure, probably because TFM was more toxic at the higher temperature, but those in the second group were not affected. None of the treated adults showed signs of blindness, and all spawned normally in November. Viability and hatchability of the eggs were similar in the treated and control fish (Figure 5), and growth of the young was not affected.

The results of these investigations serve to illustrate the utility and versatility of chronic and partial chronic toxicity tests in estimating potential impact or non-impact of pesticides on aquatic organisms. At present, there are about 30,000 registered pesticide formulations that must be

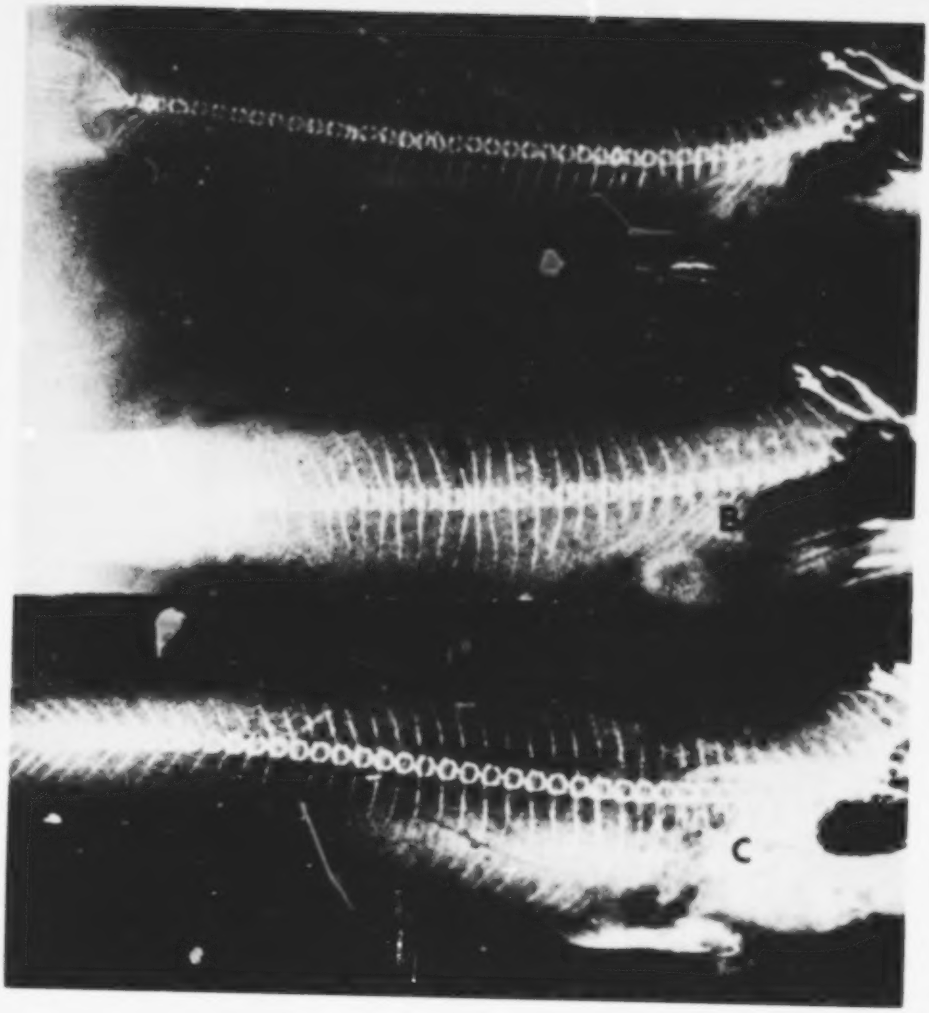

Figure 4. Effects of toxaphene on backbone structure of channel catfish. Radiograph A represents a fish exposed to 0.055 μg/liter, B a fish exposed to 0.044 μg/liter, and C a control.

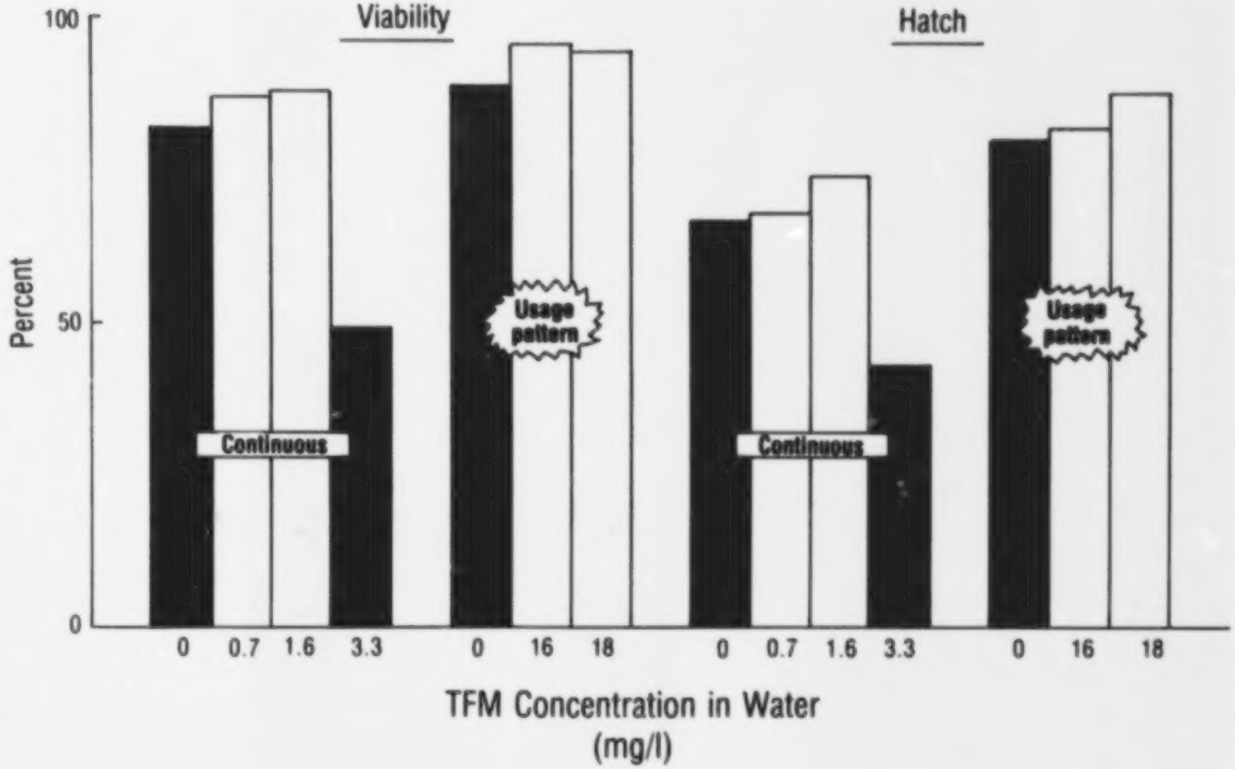

Figure 5. Comparison of viability and hatch in eggs from brook trout exposed continuously or by simulated usage pattern to TFM.

periodically re-registered. Because a significant number of new and registered pesticides may contaminate aquatic ecosystems, chronic or partial chronic tests provide an important intermediate measure of relative hazard between simple acute toxicity tests and costly experimental field trials. Within practical limits such studies can be adapted to include simulation of various general aquatic habitats, and timing and concentration of exposures can be controlled to approximate proposed or recommended uses. In addition, chronic test systems offer a unique opportunity to conduct concurrent or parallel studies of residue dynamics and physiological, biochemical, and pathological effects that can be linked to growth and reproductive effects in primary chronic tests.

SUMMARY

Requirements for registration, re-registration, and classification of pesticides for general or restricted use were published recently by the EPA. If the pesticide is intended for outdoor uses, data must generally be submitted that permit evaluation of hazards to non-target animals, including fish and wildlife. Depth of these evaluations depends on proposed patterns of use, environmental chemistry characteristics, and nature of the hazard to humans, domestic animals, and non-target animals. Data to support registration can be obtained from acute and chronic or partial chronic toxicity studies, simulated field tests, or field monitoring and observation, as described in the extensive registration guidelines recently proposed by EPA. Chronic testing techniques and apparatus, with controllable light and temperature, offer versatile systems for investigating effects of pesticides and other contaminants on fish according to daily and seasonal periodicity and simulated pesticide-use patterns.

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SECTION 17

EXPERIMENTAL RESEARCH ON PHENOL INTOXICATION OF AQUATIC ORGANISMS AND DESTRUCTION OF PHENOL IN MODEL COMMUNITIES

M.M. Kamshilov and B.A. Flerov[1]

In the first part of the investigation, some particular and general problems of aquatic toxicology have been studied on a model of phenol intoxication of aquatic organisms. Among the problems investigated were comparative resistance of aquatic organisms, the role of biotic and abiotic factors in determining, resistance, effects of different concentrations of the toxicant on biological and physiological processes of aquatic organisms, and ability of organisms to adapt. In the second part of the investigations, destruction of phenol in different model ecosystems has been studied. Workers from the laboratory of the physiology of lower organisms took part in the investigation. (V.A. Alekseyev, L.A. Baronkina, P.A. Gdovskiy, N.V. Goryacheva, B.F. Zhukov, L.I. Zakharova, V. Ya. Kostyayev, N.A. Lapteva, G.A. Lukina, V. Ye. Matey, F.I. Mezhnin, V.R. Mikryakov, T.F. Mikryakova, G.E. Flerova).

The principal results of the first part of the investigations are presented in the following text.

BACTERIA

Phenol, even in small concentrations (10 mg/liter) produces an inhibitory effect on bacteria of the genera Bacterium, Cornybacterium, and Micrococcus. Bacteria of the genera Pseudomonas and Micobacterium were more resistant to the toxicant: At a concentration of 50-200 mg/liter, an increased development took place. At a concentration of 1000 mg/liter, phenol exerts bacteriostatic effect on these genera.

ALGAE

The most resistant algae are green algae. Retarding their growth takes place at 30-60 mg/liter, complete inhibition at 300-600 mg/liter. Least resistant are the chrysophyte algae. Complete inhibition of their growth is observed at 8-15 mg/liter. According to resistance, green algae and diatoms are intermediate. In blue green algae, reproduction ceases at 100 mg/liter and in diatoms at 200 mg/liter.

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[1] Institute of Biology for Inland Waters, Acad. Sci. USSR.

Complete inhibition of photosynthesis in all algae occurs at concentrations from 700 to 1400 mg/liter. Resistance of algae to phenol is to a considerable degree determined by the composition of the medium. The richer the medium in nutrients, the more resistant are the algae grown on it.

Chlorella has been studied in more detail. Inhibition of its growth commences at 100 mg/liter. At 1500 mg/liter reproduction ceases. The toxic effect of phenol is directly proportional to the light intensity. Resistance of various strains of Chlorella is determined by their sensitivity to light. Respiratory processes in Chlorella are more tolerant to the influence of phenol than photosynthetic processes.

INVERTEBRATES

Different crustaceans, molluscs, aquatic insects and arachnids were used as experimental organisms. Resistance of invertebrates to phenol varied widely: At 48 hrs exposure and a temperature of 20 C, LC50s fluctuate from 2 to 2000 mg/liter; however, the organisms may be divided into three groups (Table 1) according to their resistance.

There are low resistance invertebrates--larvae of caddis flies (genus Trichoptera), stoneflies (order Plecoptera), mayflies (order Ephemeroptera), beetles (order Coleoptera), damselflies (order Odonata), blackflies (order Simuliidae) and crustaceans (suborder Cladocera). Their LC50 are in the range of 2-50 mg/liter.

Invertebrates of intermediate resistance are larvae of culicidflies (family Culicidae, Subfamily Orthocladiinae), order Megaloptera and image bugs (genera Sigara, Gerris). Their LC50s are in the range of 50-300 mg/liter.

There are highly resistant invertebrates--larvae of other flies (with the exception of the families Simuliidae, Culcidae, subfamily Orthocladiinae), image bugs (with the exception of the genera Sigara, Gerris), of beetles, molluscs, spiders and mites. Their LC50 are in the range of 400-2000 mg/liter. Aquatic invertebrates with respect to their resistance to other toxic substances (pesticides) are arranged approximately in the same order.

A comparison of the resistance of organisms indicates that Sida crystallina from the Cladocera family may serve as a susceptibility test-object for toxicological investigations. This organism, like Daphnia, is easy to rear under laboratory conditions.

TABLE 1. RESISTANCE OF AQUATIC INVERTEBRATES TO PHENOL

Degree of Resistance	Species	48 hrs LC50 mg/l
Low Resistant	TRICHOPTERA (larva)	
	Limnophilus flavicornis Ebr.	2
	Leptocerus aterrimus Steph.	2
	Phryganea striata L.	2
	Limnophilus stigma Curt.	7
	EPHEMEROPTERA (larva)	
	Baetis sp.	2
	Cloeon dipterum L.	5
	Siphlonurus linnaeanus Eat.	22
	PLECOPTERA (larva)	
	Nemura marginata Pict.	7
	COLEOPTERA (larva)	
	Acilius sulcatus L.	16
	Ilybius angustior Gyll.	46
	Dytiscus marginalis L.	46
	ODONATA (larva)	
	Platyckemis pennipes Pall.	24
	Coenagrion pulchellum V.d.L.	28
	Lestes dryas Kir.	30
	Aeschna cyanea Mull.	30
	Sympetrum flaveolum L.	30
	DIPTERA (larva)	
	Ensimulium ex. gr. aureum Fries.	16
	CLADOCERA	
	Sida crystallina O.F. Muller.	6
	Daphnia longispina O.F. Muller.	18
	Chydorus sphaericus O.F. Muller.	20
	Daphnia pulex De Geer.	36
	Bosmina coregoni Baird.	36
	Ceriodaphnia pulchella g. Sars	42
	Lynceus brachyurus O.F. Muller.	47
Intermediary Resistant	DIPTERA (larva)	
	Aedes caprius Ludl.	50
	Cryophila lapponica Mart.	50
	Mochlonyx culiciformis De Geer	50
	Orthocladius sp.	100
	Anopheles maculipennis Meig.	190
	Chaoborus crystallinus De Geer.	240

TABLE 1. RESISTANCE OF AQUATIC INVERTEBRATES TO PHENOL (con't.)

Degree of Resistance	Species	48 hrs LC50 mg/l
Intermediary Resistant	MEGALOPTERA (larva)	
	Sialis flavilatera L.	280
	HEMIPTERA (image)	
	Sigara striata L.	165
	Gerris lacustris L.	200
Highly Resistant	DIPTERA (larva)	
	Ablabesmyia monilis L.	400
	Chironomus plumosus L.	530
	Psectrocladius ex gr. psilopterus Kieff.	830
	Trigoma sp.	830
	Eristalis sp.	2000
	HEMIPTERA (image)	
	Notonecta glauca L.	450
	Naucoris cimicoides L.	500
	COLEOPTERA (image)	
	Haliplus flavicollis Sturm.	440
	Hydrobius fuscipes L.	860
	Gyrinus marinus Gyll.	1000
	Coelambus novemlineatus Steph.	1000
	Ilybius angustior Gyll.	1000
	Dytiscus marginalis L.	1800
	BIVALVIA	
	Dreissena polymorpha Pall.	1000
	Anodonta piscialis Niles.	1000
	Unio pictorum L.	1000
	Unio tumidus Phill.	1000
	Sphaerium corneum	1000
	ARANCINA (image)	
	Argyroneta aquatica Cl.	1500
	ACARIFORMES (image)	
	Hygrobates longipalpis Herm.	440
	Hydrachna merita Wainst.	440
	Limnesia undulata Mull	660
	Piona nodata Mull.	660
	Eylais hamata Koen.	660
	Limnesia maculata Mull.	900
	Hydrodroma despiciens Mull.	1180

TABLE 1. RESISTANCE OF AQUATIC INVERTEBRATES TO PHENOL (con't.)

Degree of Resistance	Species	48 hrs. LC50 mg/1
Highly Resistant	*Piona coccinea* Koch	1500
	Hydryphantes ruber De Geer.	1680
	Limnochares aquatica L.	1560
	Mideopsis orbicularis Mull.	1720
	Arrhenurus globator Mull.	1840

The difference in resistance of aquatic invertebrates is determined by many factors. The most important of these are morpho-physiological (size of external coverings, their permeability, peculiarities of their respiratory system, their surface) and behavioral (ability to avoid toxicant, general activity in a toxic environment) characteristics.

When there are sublethal concentrations (less 5 mg/liter) on Daphnia longispina, behavior and ^{14}C trace food consumption is initially disrupted, and later on embryogenesis and fertility are disrupted. Thus, physiological indices in intertebrates are good specific tests for water toxicity.

FISH

Three stages of phenol intoxication in fish have been observed: (1) disorderly general motor activity, (2) loss of equilibrium and, (3) cessation of motor activity and respiration. The main stages of poisoning are the same for freshwater fish, but the degree of manifestation and duration of intoxication varied in different species of fish.

The species characteristics of susceptibility (initial reaction to the action of the toxicant) and resistance of freshwater fish to phenol have been found.

Regarding susceptibility, the fish species may be arranged in the following order of increasing sensitivity--crucian carp, blue bream, burbot, bream, perch, pike, ruffe, roach, trout. Regarding resistance, the following descending order is observed--crucian carp, roach, bream, blue bream, pike, ruffe, perch, burbot, trout. It has been observed that high susceptibility did not always correlate with low resistance and vice versa. For example, roach (Rutilis rutilis L.) is highly susceptible and highly resistant; burbot (Lota lota L.), is on the contrary, has low susceptibility and low resistance.

Fry are the most resistant, mature fishes are the least. Differences in fish age are leveled with an increase in concentrations. Resistance to phenol decreases with significant increase in body weight. Resistance of fish to a toxicant is considerably less in summer than in winter.

The role of basic environmental factors in fish resistance to phenol has been demonstrated. Resistance of fish falls with a decrease in the dissolved O_2 content and with an increase in temperature. Water hardness and pH influence the resistance of fish to phenol practically not at all.

The adaptation of fish to the toxicant has also been investigated. Fish preliminarily exposed to sublethal concentrations of phenol for different time periods (from a week to more than a year) when put into a solution with extremely toxic concentrations showed a lesser resistance to lethal concentrations than those not exposed. This proves that a fish can not individually adapt to phenol. The adaptation is realized by selecting the most resistant individuals. The first generation of fish (guppies) was already 5 times as resistant as the initial generation. The selection is of a nonspecific character. After the selection for resistance to phenol, the fish were simultaneously more resistant to another toxicant, polychlorpinene (Figure 1).

We have also investigated the effect of sublethal concentrations on different vital biological characteristics of fish: Behavior (general moto activity, feeding, defense, sexual behavior, conditioned responses) growth rate, and reproduction. Physiological functions such as bioelectric activity of nerve and muscle systems, neurosecretion in the hyothalamo-hypophysial system, immunological reactions, and blood analysis have been included as indices of intoxication. Pathologic changes in the structure of fish organs have been studied under lethal concentrations.

Chronic phenol influence causes considerable changes in behavior, and first of all in conditioned reflexes and then in other functions of the organism (Figure 1). Conditioned reflex method may be recommended as a highly sensitive test for determining water toxicity. Under a prolonged toxicant influence, inhibition of feeding, sexual behavior, defense functions, and growth rate takes place.

After Kuba's (1969) investigations, it has been established, on the basis of electrophysiological data, that phenol acts primarily on neuromuscular synapses, increasing the frequency in formation of miniature terminal disc potentials (Figure 2). Concentrations of phenol lethal for the whole organism do not produce a noticeable effect on the total action potential of peripheral nerves nor on the evoked responses of the fish brain (olfactory bulb).

Pathomorphological changes in fish organs and hypothalamo-hypophysial system reactions under phenol intoxication are of general and nonspecific character. Thus, they are unlikely to be of any significance for postmortem diagnosis to toxicosis.

The principal results of the second part of the investigation are presented below.

DESTRUCTION OF PHENOL IN MODEL COMMUNITIES

The destruction of phenol was studied in 28 aquaria, 3-15 liter in volume, filled with river water and sand. Several components (aquatic organisms, mineral fertilizers, ultraviolet radiation) were introduced into the aquaria.

One series of experiments performed in 6 aquaria may be cited as an example.

Aquarium 1 - without aquatic organisms

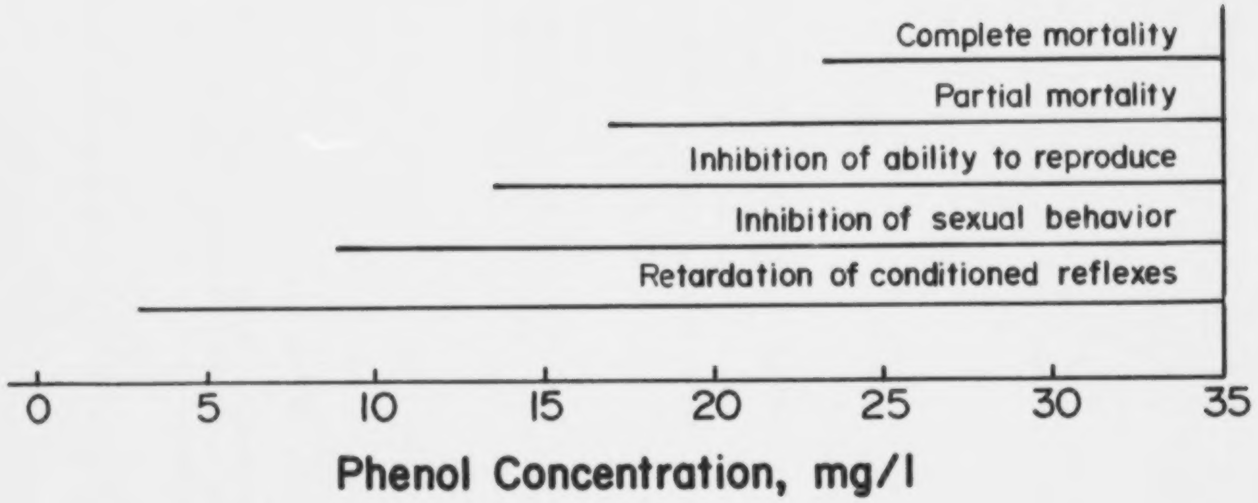

Fig. 1. Sequence of changes in biological indices of Lebistes reticulatus (P) in phenol concentrations.

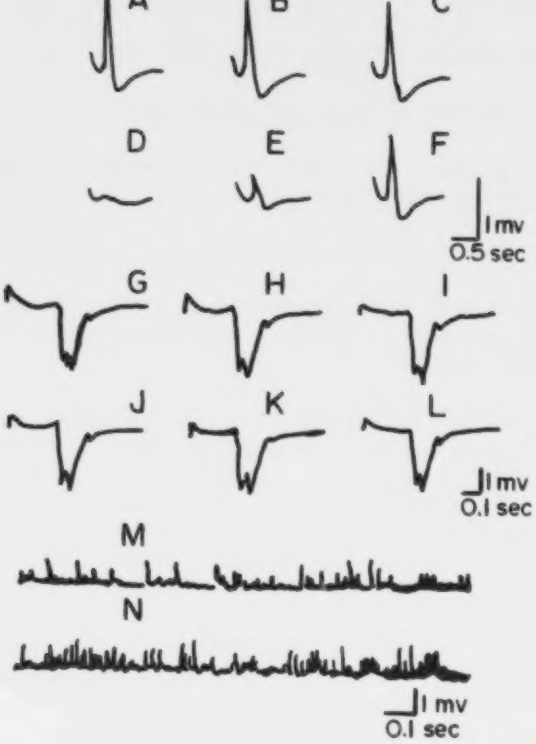

Fig. 2. Effect of phenol on bioelectrical activity of peripheral, central nerve system and neuromuscular conjunction of fisn.

A-F Influence of phenol on action potential of olfactory nerve in pike.
A-control, B-500 mg/l, C-1000 mg/l (10 min), D-2000 mg/l (1 min), E-wash out by Ringer solution (5 min), F-wash out by Ringer solution (15 min).

G-L Resistance to phenol of the potential generated by olfactory bulb in pike (brain membrane is removed).
G-control, H-10 mg/l, I-100 mg/l, J-500 mg/l, K-1000 mg/l, L-wash out.

M,N Influence of phenol on miniature potentials of terminal discs in the red muscle of the pectoral fin in the carp.
M-control, N-5 min after application of mg/l of phenol.

2 - with filamentous algae (mougeotia genuflexa) and oligochaets (Lumbriculus variegatus).

3 - with duckweed (Lemna trisulca).

4 - with elodea (Elodea canadensis).

5 - with elodea, duckweed and molluscs (Limnea stagnalis and Planorbis sp.).

6 - with the same content as in 5.

It is quite natural that some microscopic organisms were introduced together with the macrocomponents (Elodea, duckweed, molluscs, oligochaets) and with the water and sand.

Phenol was added to the five aquaria first in doses of 1 mg/liter (1st period - 59 days), then in doses of 5 mg/liter (2nd period - 112 days), and then in doses of 10 mg/liter (3rd period - 194 days). Altogether, 2388 mg of phenol per liter of medium were added to all the experimental aquaria during the three periods (365 days). A year after the beginning of the experiment, the addition of phenol was stopped (4th period - 140 days). Aquarium #6 was the control; phenol was not added to it.

The concentration of phenol was systematically measured by pyramidon method (Kaplin and Fesenko, 1962). The contents of nitrogen, phosphorus, oxygen, pH and BOD_5 in the medium were determined less regularly. The bacterial population of the aquaria, especially the number of saprophytic bacteria decomposing phenol, was constantly controlled. The numbers and species composition of colorless flagellates, infusoria, algae and fungi were also determined.

As seen in Figure 3 which presents data on accumulation and decomposition of phenol, the destruction of this toxicant takes place faster in the aquarium having the most diverse composition (5) than in other aquaria.

The results of the second section of the research allows us to draw the following conclusion.

The rate of phenol destruction is, given other similar conditions, a function of the diversity of the community taking part in the destruction process. The living population of a water-body is able to cope with external disturbances, acting as a self-regulating system only if it is diverse enough. The basis of the self-regulation is biotic circulation, i.e., the same processes which guarantee the yearly repeated cycles of biotic production. The presence of oxygen and nutrients (nitrogen salts and phosphorus), being a very important factor in the destruction of phenol, is by itself not sufficient to guarantee its effectiveness. A very energetic decomposition of the toxicant may occur at relatively low values of these factors.

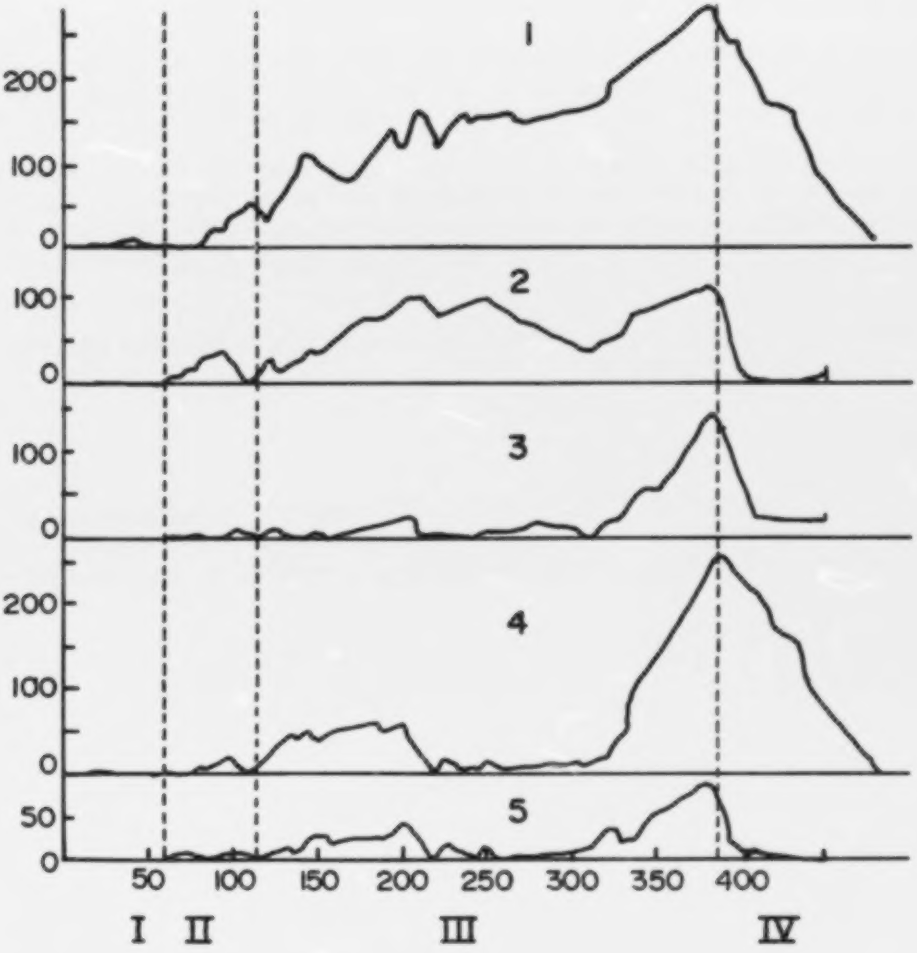

Fig. 3. Accumulation of phenol in model communities.

Abscissa - days from the beginning of the experiment. Ordinate - concentration of phenol in mg/l. 1-5 - numbers of aquaria. Vertical broken lines separate the periods differing in amount of phenol (mg) added per liter of medium: 1-1 mg/l; 2-5 mg/l; 3-10 mg/l; 4-0 mg/l. Thick continuous lines - concentration of phenol in various aquaria.

A large number of bacteria which destroy (decompose) phenol do not guarantee its active decomposition. Only the bacteria involved in biotic circulation are capable of energetically destroying the toxicant (phenol) at the minimum values of other factors (oxygen, nutrients).

When a toxic substance is added regularly in small portions, a biological system is able to decompose it in much greater quantitities than when the same toxicant is added at once in a large quantity.

To create highly effective detoxicating systems of living organisms, it is necessary to account for an adaptation period during which complexes of organism species, capable of effectively decomposing the toxicant, are established. The duration of the adaptation period is approximately two months.

When the toxicant is no longer introduced, the ecosystem quickly loses its ability to decompose it.

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SECTION 18

HISTORY OF CHANGES IN FISH SPECIES OF THE GREAT LAKES

John F. Carr

INTRODUCTION

Changes began in the fish-species complex in the Laurentian Great Lakes almost immediately after the first permanent settlers arrived in the basin in the early 1800's. Changes occurred slowly at first, but accelerated with the increased activities of man. These changes continue today and will continue in all probability for decades or even centuries because man's manipulations of the environment are continuing.

The Great Lakes are young; only about 10,000 years have passed since the melting of the glaciers. Youthful lakes such as these are generally characterized by low biological productivity, low nutrient content, and high transparency; they are often deep and cold. So are the Laurentian Great Lakes even today. With the exception of a few areas, the waters of the Great Lakes are of excellent quality and can be used as potable water without treatment. Yet man's impact on these lakes, especially on the fish populations, has been so drastic that the Laurentian Great Lakes have been used as worldwide symbols of accelerated aging. Some scientists have estimated that the lakes, especially Erie and Ontario, have aged more in the past 150 years than in the preceding 10,000 years. That changes of this magnitude could occur in lakes as large as the Great Lakes was not considered possible only a few years ago. Today, however, we are beginning to realize the tremendous capacity we possess to change (usually to our detriment) even the oceans and the atmosphere.

The purpose of this paper is to discuss the changes which have taken place in the fish populations of the Great Lakes and the stresses which have caused these changes. It has become obvious that many of the causes of the declines are the results of deliberate actions rather than subtle unpredictable factors.

The stresses which have been placed on the fish communities of the Great Lakes have been sequential and reflect the progress of man's occupation of the basin and his technological development. The most obvious and primary direct stress has been the intensive and selective exploitation of the fish stocks. This stress began early in the 19th century and continues to some degree today. Environmental stresses have not been as direct or as obvious, but were present as early as 1830 and have been additive as well as continuous. Environmental stress on the fish of the Great Lakes has been of five general types:

(1) Physical stress resulting from modification of the watershed by deforestation, blockage of tributaries, and drainage of marshes. This primarily affected anadromous species and began during the period of low human population density.

(2) Biological stress caused by the introduction and colonization of exotic species. Introduction of new species began before 1900 and continues today.

(3) Chemical stress (first phase) in the form of oxygen-consuming organic material dumped into tributaries and bays, and increased plant nutrients in the inshore areas.

(4) Chemical stress (second phase) caused by toxic chemicals such as chlorinated hydrocarbons and heavy metals.

(5) Thermal stress - more a future concern than a present concern.

The direct effects of the environmental changes on the fish populations are seldom observed and perhaps rarely occur. Indirect effects of these changes are often cited, but only occasionally quantified. In lakes as large as the Great Lakes, cause and effect are separated in time and distance to an extent that only after an event can the two be linked. This is the situation with the continuous change in the abundance of Great Lakes fish species.

We all recognize the fact that the stresses, be they exploitation, destruction of spawning grounds, oxygen depletion, increased water temperature, change in available food, or competition with introduced species, cannot often be isolated and analyzed separately. This paper is a summary of the changes that have occurred in the fish communities of the Laurentian Great Lakes from the early 19th century to the present. The changing composition of fish populations in the Great Lakes has been the subject in recent years of many articles in scientific and popular publications. The most exhaustive discussion of these changes occurred in a recent (1971) international symposium on "Salmonid Communities in Oligotrophic Lakes" (SCOL). These papers were published as a special issue of the Journal of Fisheries Research Board of Canada in 1972. This publication contains seven papers exclusively on the Great Lakes, including case histories of each of the five Laurentian Great Lakes: Superior, Huron, Michigan, Erie, and Ontario (Figure 1). Other comprehensive papers on changes in Great Lakes fish species are by Smith (1964, 1968) and Christie (1974).

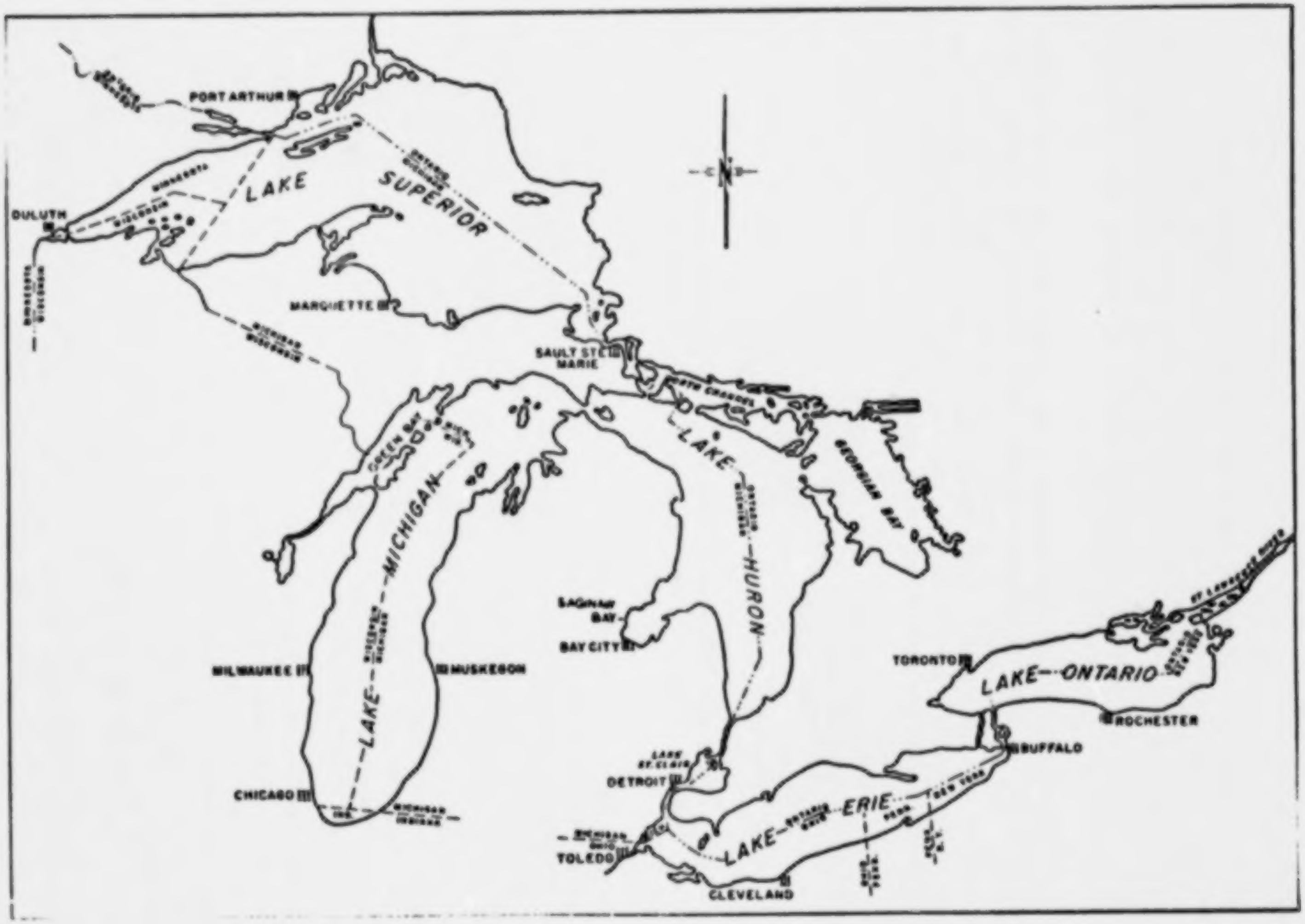
PORT ARTHUR
LAKE SUPERIOR
DULUTH
MARQUETTE
SAULT STE. MARIE
GEORGIAN BAY
LAKE HURON
LAKE MICHIGAN
GREEN BAY
MILWAUKEE
MUSKEGON
SAGINAW BAY
BAY CITY
CHICAGO
LAKE ST. CLAIR
DETROIT
TOLEDO
LAKE ERIE
CLEVELAND
BUFFALO
TORONTO
LAKE ONTARIO
ROCHESTER
NEW YORK
N

Figure 1. The St. Lawrence Great Lakes with interstate and international boundaries.

IN THE BEGINNING (TO 1850)

Fish Communities

The fish-species complex in the Great Lakes has changed drastically. Unlike many large lakes of the world, especially the large African lakes and Lake Baikal (USSR), the Laurentian Great Lakes have had only 10 thousand years between the retreat of the glaciers and the coming of man to produce, through evolutionary forces, a complex of species that is unique to the system.

The Great Lakes system did produce a few unique species in this short period, indicating that the processes were well underway to further species diversity. The evidence for this conclusion is best illustrated by the five endemic species (Smith, 1957; Scott and Crossman, 1973), all of the subfamily Coregoninae (whitefish) in the Samonidae (salmon family). These five species listed in descending order of size were:

deepwater cisco - *Coregonus johannae*
longjaw cisco - *Coregonus alpenae*
shortnose cisco - *Coregonus reighardi*
kiyi - *Coregonus kiyi*
bloater - *Coregonus hoyi*

According to Scott and Crossman (1973) all five species were found in Lakes Huron and Michigan, four in Lake Superior, three in Lake Ontario, and one in Lake Erie.

In addition to the five endemic species of ciscos, these wider ranging species were also present: lake herring (*Coregonus artedii*); blackfin cisco (*Coregonus nigripinnus*); and shortjaw cisco (*Coregonus zenithicus*). These eight species of ciscos, together with the lake whitefish (*Coregonus clupeaformis*) and round whitefish (*Coregonus cylindraceum*), characterized the Great Lakes fish community. Most of the species of the whitefish subfamily, especially the ciscos, were inhabitants of deep, cold water and therefore reached their greatest diversity in Lakes Superior, Huron, Michigan, and Ontario (Table 1). The dramatic alteration in the species complex of deepwater ciscos that subsequently occurred was documented by Smith (1964) for Lake Michigan.

In addition to the Coregonines other groups and species were abundant in the lakes. The dominant predators of the open waters present in all five lakes were the lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*). In the bays and nearshore areas were: lake sturgeon (*Acipenser fulvescens*); northern pike (*Esox lucius*); suckers (primarily *Catastomus catastomus* and *C. commersoni*); channel catfish (*Ictalurus punctatus*); bullheads (*Ictalurus* spp.); white bass *Morone chrysops*; freshwater drum (*Aplodinotus grunniens*); and three species of the perch family: yellow perch (*Perca flavescens*); walleye (*Stizostedion vitreum*); and sauger (*Stizostedion canadense*). All of these species have been historically of commercial significance. The Atlanta salmon (*Salmo salar*) and American eel (*Anguilla rostrata*) were also abundant and became commercially important only in Lake Ontario.

TABLE 1. DIMENSIONS OF THE GREAT LAKES[a]

Lake	Length (miles)	Breadth (miles)	Water surface (miles)	Drainage basin (miles)	Average surface elevation above mean sea level since 1860 (ft)	Mean discharge (cfs)	Maximum depth (ft)	Mean depth (ft)
Superior	350	160	31,820	80,000	602.20	73,300	1,333	487
Michigan	307	118	22,400	67,860	580.54	55,000	923	276
Huron	206	183	23,010	72,620	580.54	177,900	750	195
St. Clair	26	24	490	7,430	574.88	178,000	21	10
Erie	241	57	9,930	32,490	572.34	195,800	210	58
Ontario	193	53	7,520	34,800	246.03	233,900	802	283

[a] From Beeton and Chandler, 1963.

Knowledge of the presence and relative abundance of the species listed thus far is based on records of the harvest of these species by commercial fishermen. Species not of great commercial value were, of course, also present in the lakes. A complete list of all species known to have been present in the lakes would be too long to include for the purposes of this paper. Our knowledge of changes in abundance of a few of these species, however, is sufficient to warrent their inclusion. An abundant forage species in the deeper water of all the lakes was the fourhorn sculpin (Myoxocephalus quadricornis). The slimy sculpin (Cottus cognatus) inhabited intermediate depths in all lakes. The inshore waters contained a variety of species of several families, especially the Cyprinidae. Among the more abundant species were the emerald shiner (Notropis atherinoides) and the spottail shiner (Notropis hudsonius).

Thus, when settlement began in the first half of the 19th century, the lakes were occupied by a supposedly stable community of fish species which inhabited all niches from the deepest waters of the subartic Lake Superior to the shallow bays and marshes of Lake Erie.

Environmental Conditions

Physiochemical conditions of the lakes were not measured before the end of the 19th century. Beeton and Edmondson (1972) used as a basis for evaluating the "natural" chemical condition the limited chemical data available about 1900 as indicative of the pristine quality of the lakes (Table 2).

TABLE 2. ESTIMATED AVERAGE CONCENTRATION OF DISSOLVED CHEMICAL CONSTITUENTS IN THE GREAT LAKES PRIOR TO 1900 (EXPRESSED IN MG/LITER)[a]

Lake	Total dissolved solids	Calcium	Sulphate	Chloride	Sodium and potassium
Superior	60	13	4	2	3
Michigan	128	34	5	2	-
Huron	108	24	6	4	4
Erie	142	31	13	7	7
Ontario	140	31	15	7	6

[a] From Beeton, 1969.

Based on these few constituents, Lakes Michigan, Erie, and Ontario were similar in chemical composition (Table 2). Lake Superior's chemical levels were substantially below all the others. Lake Huron receives approximately 41% of its inflow from Lake Superior, 31% from Lake Michigan, and 28% from the Lake Huron basin. Apparently the basin is a major contributor to the characteristics of the water quality, because the total dissolved solids are much higher than would be expected based only on a mixture of the waters from Lakes Superior and Michigan. The Lake Erie watershed apparently contributed significant amounts of calcium, sulphate, chlorides, and sodium-potassium to the waters flowing into Lake Ontario, because their chemical characteristics are nearly identical. Although differences between lakes were large compared to the range between other natural bodies of water, the five Great Lakes were remarkably similar.

Analysis of nutrient concentrations for the Great Lakes has been made only in recent years; therefore, base levels are only now being established. Estimates based on recent values for phosphorus and nitrogen indicate that the levels in the mid-1800's were less than 10 μg/liter for phosphorus and usually less than 1 mg/liter for total nitrogen in the three upper lakes (Superior, Huron, and Michigan). The Great Lakes in the 1900's would have been classified as oligotrophic (as defined by Hutchinson, 1957) with the probable exception of Lake Erie.

The order of the lakes, if listed from the greatest to the least fishery productive potential in the 1800's, probably would be Lake Erie, Lake Ontario, Lake Michigan, Lake Huron, and Lake Superior (Figure 2).

THE INITIAL IMPACT OF SETTLEMENT (1850-1900)

Changes in Fish Populations

The first environmental stresses on the Great Lakes ecosystem were primarily caused by physical alterations in the basin, particularly in the lower lakes. These alterations were deforestation of the watershed and siltation and blockage of streams. These changes mainly affected the tributaries and consequently the obligate anadromous species. Christie (1972) reported the Atlantic salmon had begun to decline as early as 1830 in Lake Ontario and was extinct, or nearly so, by 1900. Documentation of the early proliferation of mills and dams was given by Christie (1972) based on data from Richardson (1944). On the Ganaraska watershed (one of the larger Canadian rivers tributary to Lake Ontario) at least two sawmills, two grist mills, and two dams had been constructed by 1800. Construction of the mills and dams increased rapidly, reaching a maximum of 34 sawmills, 19 grist mills, 4 woolen mills, and 34 dams by 1860. In 1930, 15 dams still remained on this single tributary. Christie (1972) considered the elimination of the Lake Ontario Atlantic salmon stock as the best known example of the effects of despoilation on a species habitat.

The lake sturgeon population in all the lakes was greatly reduced during this period. Prior to 1903 the annual commercial production fluctuated between 100,000 pounds and 500,000 pounds in Lake Ontario (Baldwin and

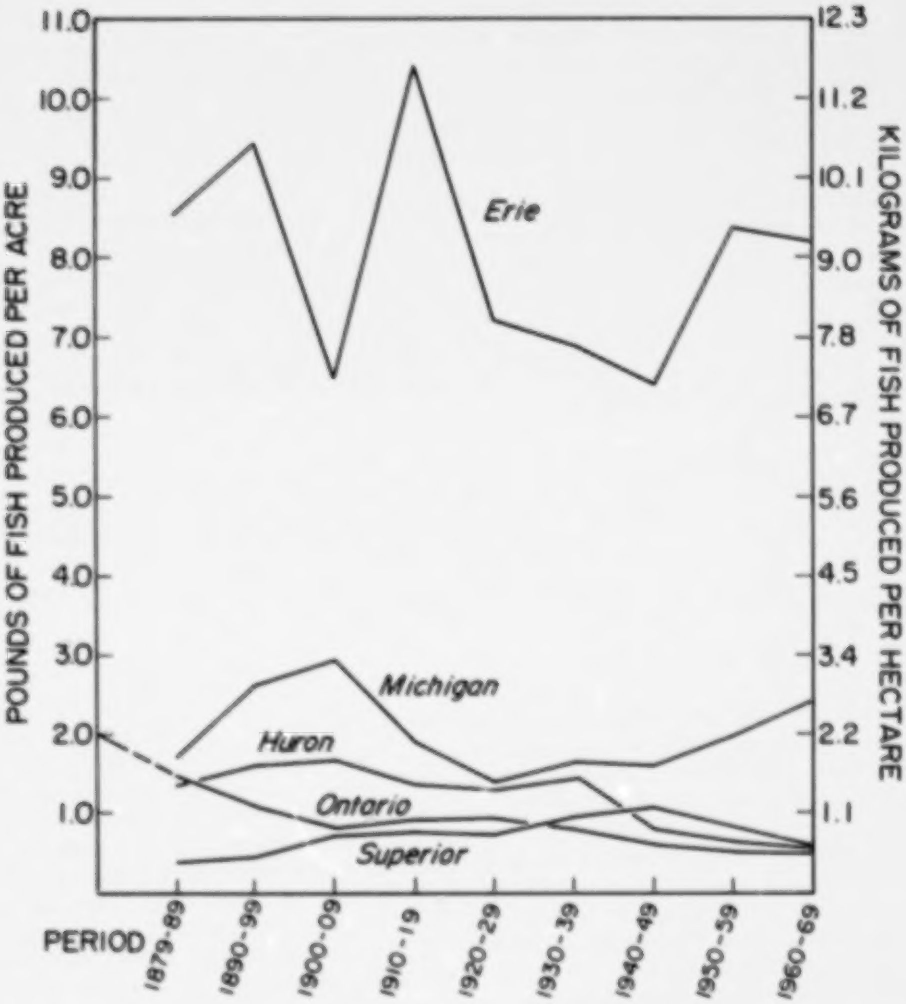

Figure 2. Average number of pounds and kilograms of fish produced per acre and hectare by the commercial fishery of the Great Lakes for 10-year intervals (data from Smith 1972).

Saalfeld, 1962). After 1910 production never exceeded 25,000 pounds and averaged near 15,000 pounds for many years. In Lake Erie production dropped from 1 - 5 million pounds in the late 1800's to less than 10,000 pounds after 1910 (Baldwin and Saalfeld, 1962). Similar magnitudes of decline occurred during the same period in Lake Huron, Michigan, and Superior. The cause-and-effect relationship apparent between watershed and stream modification and Atlantic salmon extinction was not as direct with the sturgeon. The environmental requirements of the sturgeon were not as narrow as those of the Atlantic salmon; however, the slow growth rate and late maturity of the sturgeon were also factors in this species, inability to recover from even low exploitation rates.

Christie (1972) also lists the blackfin cisco (*Coregonus nigripinnus*) as a species that became either extinct or greatly reduced in Lake Ontario before 1900. Wells and McLain (1972) infer a sharp decline in the abundance of this species in Lake Michigan in the early 1900's. The blackfin was considered commercially extinct in Lake Superior by 1910 (Lawrie and Rahrer, 1972). This species of cisco inhabited the deep, open waters of the lakes; consequently, environmental modification of the tributary and watershed was not a factor in their decline. This species was the largest of the ciscos, and selective exploitation for it was the probable cause of its decline (Wells and McLain, 1972).

The lake trout population in Lake Erie was also decimated during this period. Hartman (1972), in discussing this species in Lake Erie, states: "Perhaps the decline of the lake trout population to near extinction best illustrates the effect of essentially one stress: intensive exploitation." Apparently, environmental stress was not a factor in the decline of lake-dwelling species during the 1800's.

In addition to the effective loss of at least one species during the 1800's, several new species became abundant. Christie (1972) listed the following species as becoming established in Lake Ontario before 1900: alewife (*Alosa pseudoharengus*); gizzard shad (*Dorosoma cepedianum*); brown trout (*Salmo trutta*); carp (*Cyprinus carpio*); and the goldfish (*Carassius auratus*). Some of these species were also introduced to the other lakes during this period. There was a flourishing fishery for carp in Lake Erie by 1899, when over 3.5 million pounds were landed (Baldwin and Saalfeld, 1962).

Environmental Changes (Age of Physical Alterations)

Man's effect on the Great Lakes ecosystem in the last half of the 19th century was dramatic and permanent. He removed the forest, built dams, constructed mills, directly exploited the fish, and opened new and more direct passage between the ocean and the lakes, as well as between Lake Ontario and the upper lakes (Figure 3).

The effects of these physical modifications of the environment ranged from immediate (Atlantic salmon extinction) to long-term (invasion of marine species). The Erie Canal, which provided a connection between the Atlantic Ocean and Lake Ontario, was opened in 1819 and extended to Lake Erie in

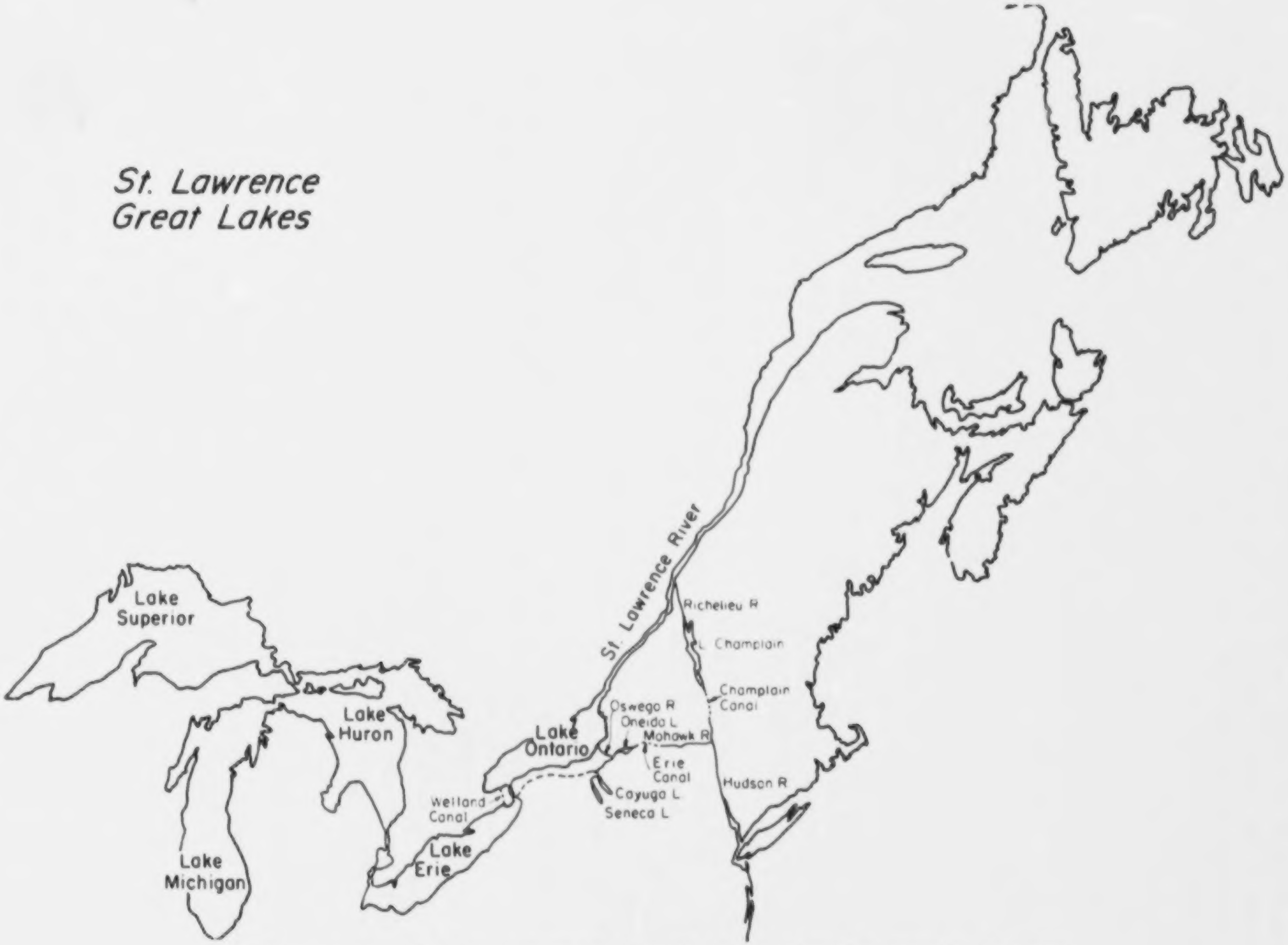
St. Lawrence
Great Lakes
Lake Superior
Lake Huron
Lake Michigan
Lake Erie
Welland Canal
Lake Ontario
St. Lawrence River
Oswego R
Oneida L
Mohawk R
Erie Canal
Cayuga L
Seneca L
Richelieu R
L. Champlain
Champlain Canal
Hudson R

Figure 3. The St. Lawrence Great Lakes showing canals between central Atlantic Ocean and the lakes.

1825. The Welland Canal, which was opened in 1829, connected Lake Ontario to the upper lakes. Previously the passage of fish between Lakes Ontario and Erie was blocked by Niagara Falls. Although no biological changes were noticed for many years after the opening of the canals, the stage was set for dramatic and catastrophic changes to occur decades later (Aron and Smith, 1971).

The chemical characteristics of the open waters of the lakes were assumed to be essentially the same at the end of the 19th century as at the beginning (Beeton and Edmondson, 1972). Although the settlement of the Great Lakes basin had advanced rapidly in the 19th century, from a population of a few thousand early in the century to over 10 million in 1900 (Table 3), the effects on water quality in the lakes were yet to be felt.

By 1900 man, in less than 100 years, had placed the following stresses on the biological communities of the Great Lakes: siltation of streams; blockage of tributaries; increase in stream temperatures; and establishment of exotic species. In addition, he had removed barriers to migration between the lakes; had established fisheries capable of overexploitation of most species in all the lakes; and had begun using the lakes as the receiver of man's domestic and industrial waste.

TABLE 3. ESTIMATED POPULATION (MILLIONS) IN GREAT LAKES BASIN - 1900-1960[a]

Lake basin	1900	1925	1950	1960
Superior	0.4 (50)[b]	0.6 (33)	0.8 (12)	0.9
Michigan	4.0 (-20)	3.2 (50)	4.8 (23)	5.9
Huron	1.0 (20)	1.2 (25)	1.5 (33)	2.0
Erie	3.0 (93)	5.8 (48)	8.6 (17)	10.1
Ontario	2.0 (25)	2.5 (20)	3.0 (33)	4.0
Total	10.4 (28)	13.3 (41)	18.7 (22)	22.9

[a] From Beeton, 1969.

[b] Numbers in parentheses indicate percentage change in the ensuing time interval.

ENRICHMENT AND INVASION (1900-1950)

The stage was set and the signs were present in 1900 for what was to follow. Changes in the biological, chemical, and physical environment of the Great Lakes became the rule and not the exception. The records of these changes, unfortunately, are incomplete, often inaccurate, and, for the fish populations, often not a true representation of species abundance. The analysis of changes in the abundance of species until recently was based on the reported catch of commercial fishermen. High prices often maintained high catches in the face of a decreasing abundance. Conversely, low production often was due to low prices and lack of demand for a species rather than low population levels. Despite these handicaps, the changing conditions often became too obvious to be ignored.

Changes in fish-species composition, losses and gains, differ in time between the lakes, but the sequence of species change often was similar (Smith, unpublished manuscript). In general, the species that declined were those most sought after by the commercial fishery. A few significant exceptions exist to this generalization, and it is the exceptions which clearly indicate stresses other than fishing on the biological communities of the Great Lakes.

Changes which occurred in native fish species of commercial interest between 1900 and 1971 are summarized in Table 4. Detailed discussion of these declines by species and lakes appear in Smith (1968), the papers of the SCOL Symposium (1972), and Christie (1974). The data presented in Table 4 refer to production trends in the total lake and, therefore, are not descriptive of events in the unique ecological areas of each lake such as the Bay of Quinte in Lake Ontario, the western basin of Lake Erie, Saginaw Bay of Lake Huron, or Green Bay of Lake Michigan (Figure 1). These geographic areas were, and are, more shallow and productive and warmer than the open portions of the lake to which they are connected. The fish-species complex here was also more diverse than in the open lake, containing many warmwater species, especially the centrarchids and percids.

Ecological and Cultural Changes, 1900-1925

During the first quarter of the 20th century, the northern pike fishery was reduced to a fraction of former production; lake whitefish in Lake Ontario and lake herring in Lake Erie began declining; the first sea lamprey was reported in Lake Erie; and the first rainbow smelt were found in Lakes Michigan and Huron. The gains and losses in these and other species were to be repeated many times in the next 50 years in the other lakes.

The introduction of the smelt into Crystal Lake in the drainage basin of Lake Michigan was deliberate, but its establishment in Lake Michigan was not contemplated, nor was its rapid spread to other Great Lakes. The sea lamprey reached Lake Erie nearly 100 years after the Welland Canal was opened and established itself in the upper lakes. The beginning of the declines in lake whitefish and lake herring were, of course, undetected at the time and thus alarmed no one.

TABLE 4. SUMMARY OF FISH SPECIES DECLINE IN THE GREAT LAKES BY YEAR, LAKE, AND CURRENT COMMERCIAL PRODUCTION

Species and lake	Beginning of decline (year)	Product first below 100,000 pounds (year)	1974 production (1,000's pounds)	Potential for recovery (H,M,L)[a] and reason
Lake trout				
Ontario	1928	1942	1	H - stocking and sea lamprey control (s.l.c.)
Erie	?	1900	0	L - environmental
Huron[b]	1935	1945	1	H - stocking & s.l.c.
Michigan	1943	1950	37	H - stocking & s.l.c.
Superior	1950	c	526	H - stocking & s.l.c.
Lake whitefish				
Ontario	1924	1966	16	L - environmental
Erie	1953	1960	1	L - environmental
Cisco (chubs)				
Ontario	1941	1950	0	L - environmental
Huron[b]	1961	1970	50	L - environmental
Michigan	1970	c	3,267	L - environmental
Superior	1965	c	1,926	L - environmental
Lake herring				
Ontario	1941	1953	32	L - environmental
Erie	1924	1958	0	L - environmental
Huron[b]	1939	1957	2	L - environmental
Michigan	1952	1963	6	L - environmental
Superior	1961	c	2,186	M - stocking
Northern pike				
Ontario	1933	1938	21	L - environmental
Erie	1914	1924	15	L - environmental
Burbot				
Ontario	1930[d]	1934[e]	0	L - environmental
Erie	1947	1961[e]	0	L - environmental
Michigan	1948[d]	1959	230[f]	H - s.l.c.
Blue pike				
Ontario	1952	1955	0	L - environmental
Erie	1955	1959	0	L - environmental
Sauger				
Erie	1945	1955	0	M - stocking
Huron	1935	1937[e]	0	L - environmental

[a] H, M, and L indicate high, medium, and low potential for recovery, respectively.

[b] Excludes Georgian Bay and North Channel.

[c] Remains above 100,000 pounds.

[d] Production normally less than 100,000 pounds.

[e] Production less than 10,000 pounds.

[f] First exceeded 100,000 pounds in 1973.

Environmental changes, except in streams and bays near centers of high human population density, were nearly undetectable in 1925 in the lakes proper. Changes had occurred, however, in Lakes Michigan, Erie, and Ontario in the few chemical constituents for which data are available (Table 5). The absolute concentrations of these constituents, even the highest levels, were well below levels of ecological or toxicological concern. The rates of change, especially in total dissolved solids, sulphur, and chloride, however, are staggering considering the tremendous volume of water which had been changed as much as 160% in only 25 years.

TABLE 5. ESTIMATED AVERAGE CONCENTRATION OF DISSOLVED CHEMICAL CONSTITUENTS IN THE GREAT LAKES IN 1925 (EXPRESSED IN MG/LITER)[a] WITH PERCENTAGE CHANGE AFTER 1900 IN PARENTHESES

Lake	Total dissolved solids	Calcium	Sulphate	Chloride	Sodium and potassium
Superior	58 (-3)	13 (0)	4 (0)	2 (0)	3 (0)
Michigan	143 (+12)	34 (0)	13 (+160)	4 (+100)	-
Huron	108 (0)	24 (0)	9 (+50)	4 (0)	4 (0)
Erie	146 (+3)	33 (+6)	16 (+23)	11 (57)	7 (0)
Ontario	149 (+6)	34 (+10)	18 (+20)	11 (57)	7 (17)

[a] From Beeton, 1969.

The increase in population growth in the basins of the Great Lakes was approximately 28% between 1900 and 1925 (Table 3). Growth in numbers was greatest in the basins of Lakes Erie and Ontario. The population in the Erie basin increased 93% to 5.8 million, and in the Lake Ontario basin the increase was 25% to 2.5 million. Lake Michigan's basin effectively lost 0.8 million when the Chicago Sanitary Canal, which diverted the waste from the city to the Mississippi River drainage, was completed in 1900. The increasing urbanization and industrial expansion was the probable cause of the increase in the chemically conservative ions. Undoubtedly, concentrations of other chemical components also increased, especially the plant nutrients phosphorus and nitrogen. The load of oxygen-demanding organic compounds can also be assumed to have increased.

The stresses causing the decreases in some native fish species in the lower lakes by 1925 were man caused, principally by heavy exploitation. The role of environmental change, especially water-chemistry change, in reduction in fish populations apparently was minor except in the tributaries and bays. The drainage of marshes, however, may have been a significant factor in the loss of the northern pike as an important commercial species in Lake Erie. The establishment of the two marine species, smelt and lamprey, was too recent to have had a measurable impact on other fish species by 1925.

Environmental and Cultural Changes, 1925-1950

Several catastrophic events affecting the Great Lakes fish stocks occurred during this period. The most damaging event was the invasion by the parasitic sea lamprey of all the upper Great Lakes. After at least 50 years in Lake Ontario, the lamprey made its way into Lake Erie in 1923 where it did not flourish because of the lack of suitable spawning streams and limited deepwater environment. In 1932 the first sea lamprey was reported in Lake Huron; 4 years later the lamprey was in Lake Michigan; and by 1946 the first report was made of a sea lamprey in Lake Superior (Table 6). Three years after the first sea lamprey was reported in Lake Huron, the production of lake trout started to decline (1935), and by 1946 (Table 4) the commercial fishery for this species in Lake Huron proper was finished, although the fishery in Georgian Bay lasted another 9 years. Lake trout production began to decline in Lake Michigan in 1943 (7 years after the first lamprey was reported); by 1950 production dropped below 0.1 million pounds (Table 4), and the species was virtually extinct 3 years later. Only 18 years passed from the time the first sea lamprey was reported in Lake Huron (1932) until the species was commercially extinct in Lakes Huron and Michigan.

The demise of the lake trout population in Lake Ontario and the role of the sea lamprey is more complicated than in the upper lakes. Whether the sea lamprey was endemic to Lake Ontario (Christie, 1972), or became established after the opening of the Erie Canal (Smith, 1974), at least 75 years passed before the lake trout production began its final decline (1928). A substantial fishery continued, however, for another 10-12 years. The species was last reported in the commercial catch statistics as late as 1964 (Baldwin and Saalfeld, 1962, with supplement). That the sea lamprey was a strong factor in the loss of lake trout in Lake Ontario is undisputed; the reasons why the struggle lasted so long remain a subject of speculation.

The sea lamprey's favored prey was the lake trout, but other species as well were victims of this marine invader. Larger individuals of lake whitefish, ciscos, lake herring, suckers, and burbot were attacked by the sea lamprey. The production of burbot (never a prime commercial species) began to decline in Lake Ontario in 1930, in Lake Erie in 1947, and in Lakes Huron and Michigan by 1948 (Table 4). The burbot population became commercially extinct in these lakes about 1960.

Other species also began declining during this period, although the declines were not related to the sea lamprey. The sauger began declining in Lake Huron (primarily Saginaw Bay) in 1935; 2 years later the species was commercially extinct. Beeton (1969) gave the reason for the decline as the development of an environment not suitable for the sauger or the Saginaw

TABLE 6. FISH-SPECIES INTRODUCTIONS IN THE GREAT LAKES BY YEAR AND LAKE, FIRST YEAR OF COMMERCIAL SIGNIFICANCE, AND CURRENT PRODUCTION

Species	First recorded		First reported in commercial catch		Reached commercial significance[a]		1974 Production	
	Year	Lake	Year	Lake	Year	Lake	1,000 pounds	lake
Sea lamprey[b]	1800's	Ontario						
	1921	Huron						
	1936	Michigan						
	1946	Superior						
Alewife[b]	1873	Ontario	?		1920's	Ontario	1,332	Erie
	1931	Erie	?					
	1933	Huron	?					
	1949	Michigan	1956	Michigan	1957	Michigan	45,508	Michigan
	1953	Superior						
Gizzard shad	1900	Ontario						
Coho salmon	1968	Ontario						
	1968	Erie						
	1967	Huron						
	1966	Michigan						
	1966	Superior						
Chinook salmon	1969	Ontario						
	1970	Erie						
	1967	Huron						
	1967	Michigan						
	1967	Superior						
Rainbow smelt[b]	1923	Michigan	1946	Ontario	1952	Ontario	110	Ontario
	1925	Huron	1946	Erie	1952	Erie	15,766	Erie
	1930	Superior	1935	Huron	1950	Huron	215	Huron
	1931	Ontario	1931	Ontario	1933	Michigan	1,748	Michigan
	1935	Erie	1935	Superior	1956	Superior	2,853	Superior
Carp	1885-1895	Ontario	1899	Ontario	1910	Ontario	411	Ontario
	1880-1895	Erie	1892	Erie	1893	Erie	3,152	Erie
	1880's	Huron	1899	Huron	1908	Huron	739	Huron
	1880's	Michigan	1893	Michigan	1903	Michigan	3,244	Michigan
	?	Superior						
Goldfish	?		1929	Erie	1933	Erie	54	Erie
White perch	1950[c]	Ontario	1955	Ontario	1964	Ontario	371	Ontario

[a] Production exceeded 100,000 pounds.

[b] Year of record from Smith, 1972.

[c] Year of record from Christie, 1972.

TABLE 7. FISH-SPECIES IN THE GREAT LAKES THAT HAVE EXPERIENCED SEVERE DECLINES, LAKE AFFECTED, AND SUSPECTED CAUSE OF DECLINE

Species	Principal lakes affected	Primary cause(s) of decline
Atlantic salmon	Ontario	Deterioration and blockage of streams, exploitation
Sturgeon	All lakes	Exploitation, destruction of spawning streams
Lake trout	Erie	Exploitation
Northern pike	Erie, Ontario Huron	Destruction of spawning areas, exploitation
Lake herring	All lakes	Exploitation, environmental changes, competition with introduced species
Burbot	All lakes	Sea lamprey, environmental change
Cisco (chubs)	All lakes	Exploitation, competition with introduced species, sea lamprey
Sauger	Huron, Erie	Environmental change, exploitation
Lake trout	All lakes (except Erie)	Sea lamprey, exploitation
Walleye	All lakes	Environmental changes, exploitation, destruction of spawning streams
Blue pike	Erie and Ontario	Environmental changes, exploitation
Whitefish	All lakes	Environmental changes, exploitation, sea lamprey
Yellow perch	Erie, Huron, Michigan	Competition with introduced species, exploitation, environmental changes
Fourhorn sculpin	Ontario, Erie	Competition with introduced species, environmental change
Emerald shiner	Michigan	Competition with introduced species, environmental change

Bay populations of walleye and whitefish. In Lake Erie the sauger production fell below 0.5 million pounds in 1946 (Baldwin and Saalfeld, 1962) for the first time after nearly 70 years of production between 1 and 6 million pounds. Environmental changes, plus heavy exploitation, were believed to be the causes (Table 7).

The decline of the lake herring, historically the most productive species in the Great Lakes (Smith, 1968), began in Lake Erie in 1925, and by 1963 this fish had become commercially extinct in all the lakes except Superior. Heavy exploitation was undoubtedly a factor in the decline of the lake herring. The role and impact on this decline of introduced alewife and smelt and of environmental factors, however, have not been isolated. The collapse of the lake herring stocks in the mid-1920's was the event most responsible for stimulating interest and concern in the welfare of the Great Lakes aquatic environment. This concern was primarily responsible for identifying the rapid deterioration in the water quality of Lake Erie, which is discussed in a following section.

Water Quality and Population Changes, 1925-1950

Changes in dissolved chemical constituents continued to accelerate after 1925 in all the lakes except Superior (Table 8). The absolute values of these "indicator" chemical parameters are of no toxicological concern, but again the rate of change indicated substantial inputs from cultural and industrial sources. Concentrations of these and other chemical compounds must have been substantial in the receiving waters near the pollution source. The loss of whitefish, lake herring, sauger, and other species from the inner portions of Saginaw and Green Bays due to water quality changes would be expected.

TABLE 8. ESTIMATED AVERAGE CONCENTRATIONS OF DISSOLVED CHEMICAL CONSTITUENTS IN THE GREAT LAKES (EXPRESSED IN MG/LITER)[a] 1950 WITH PERCENTAGE CHANGE SINCE 1925 IN PARENTHESES

Lake	Total dissolved solids	Calcium	Sulphate	Chloride	Sodium and potassium
Superior	56 (-3)	13 (0)	4 (0)	2 (0)	3 (0)
Michigan	150 (5)	34 (0)	17 (31)	5 (25)	-
Huron	110 (2)	24 (0)	13 (44)	6 (50)	4 (0)
Erie	170 (16)	38 (15)	23 (44)	19 (73)	9 (29)
Ontario	172 (15)	38 (12)	25 (39)	19 (73)	10 (43)

[a] From Beeton, 1969.

Population Increases, 1925-1950

The population in the Great Lakes basin had exceeded 18 million by 1950 (Beeton, 1969), an increase of approximately 40% in 25 years (Table 3). Again, the greatest numerical growth was in the Lake Erie basin with an increase from 5.8 to 8.6 million (48%). The population in Lake Erie's basin was 46% of the total population and, combined with the Lake Ontario population, accounted for 62% of the total. The population in the Lake Michigan basin was nearly 5 million in 1950. This continued concentration of people in Michigan, Erie, and Ontario lake basins, with the associated municipal, industrial, and agricultural wastes, was the primary cause of the accelerated rate of increases in dissolved chemical constituents in these lakes.

Lake Erie--Demise is Heralded

The sudden collapse of the Lake Erie lake herring fishery in 1925 awakened the public to the need for scientific investigations into the causes of the precipitous decline. The magnitude of the decline in lake herring production was from an average of 26 million pounds per year in the previous decade to 6 million pounds in 1925, to less than a million pounds in 1929.

Since environmental factors were thought to be the cause of the lake herring decline, two intensive limnological studies (Wright, 1955; Fish, 1960) were initiated in 1928. Wright (1955) found unfavorable conditions in rivers and estuaries, but concluded that environmental changes in the open waters of the western basin of Lake Erie in 1928-30 had no adverse effect on the decline of fish stocks. Fish et al. (1960) also found no environmental basis for the decline of lake herring in the central and eastern basins in 1928-30. Although neither investigator found measurable environmental degradation in the open lake, their studies for the first time established a scientific base line of data on benthic organisms, plankton, and dissolved oxygen. The base line has subsequently been invaluable in measuring environmental changes in Lake Erie.

The effects of the sea lamprey on the lake trout stocks (previously discussed) were recognized in the 1940's, and attempts to control the lamprey began in 1946. Another decade passed, however, before an organized and substantial program was developed to control this destructive parasite.

CHANGE AND REHABILITATION (1950-1975)

Fish Stocks

Changes in abundance of fish stocks are continuing in 1975; however, many changes are now deliberate and controlled. Uncontrollable changes in native species (usually decreasing in numbers) and in introduced species (usually increasing in numbers) frequently occurred during the past 25 years.

1950's

Lake trout production reached zero in Lake Michigan and began to decline in Lake Superior in 1950. Walleye production started to decline in 1950 in Lake Michigan. Cisco (chub) production dropped below 100,000 pounds in Lake Ontario, the first of the lakes to lose its chub population.

In 1952 production of lake herring in Lake Michigan and blue pike in Lake Ontario began their "terminal" decline. Lake whitefish, blue pike, and walleye production began declining in Lake Erie by 1956. By 1959 production had fallen below 100,000 pounds for lake herring in Lake Ontario, Huron, and Erie; sauger in Lake Erie; and blue pike in Lakes Ontario and Erie. Within a few years the blue pike had become virtually extinct in Lakes Ontario and Erie. The emerald shiner, once exceedingly abundant, became extremely scarce in Lakes Michigan and Huron.

Increases also occurred in the 1950's. Smelt production exceeded 200,000 pounds in Lakes Ontario and Huron; 800,000 pounds in Lake Superior; 6 million pounds in Lake Erie; and 9 million pounds in Lake Michigan. The rainbow smelt had become a significant species in the commercial catch of all the lakes in less than 30 years after its introduction in Lake Michigan.

The first alewife was reported in Lake Michigan in 1949, and the species was first reported in the commercial catch in Lake Michigan in 1956. By 1957 production exceeded 100,000 pounds, and by 1958 (9 years after it was first reported) over 1 million pounds of alewives were produced in Lake Michigan. Similar rapid colonization of the white perch occurred in Lake Ontario; only 5 years elapsed between the first record of its presence (1950) and the first report of it in the commercial catch (1955).

1960's

During this decade species of the whitefish family continued to decline in the Great Lakes. Lake whitefish production fell below 100,000 pounds in Lakes Ontario and Erie; lake herring began declining in Lake Superior and fell below 100,000 pounds in Lake Michigan; deepwater cisco (chub) production began declining in Lakes Huron and Superior. Shallow water species also declined: walleyes in Lakes Michigan and Ontario; yellow perch in Lake Michigan; and northern pike in Lake Huron. The fourhorn sculpin, once abundant in Lake Ontario, was extremely rare in the 1960's. A major decline of the fourhorn sculpin during this period was also noted in Lake Michigan (Wells and McLain, 1972).

Rehabilitation of the Fish Stocks

Biologists recognized that rehabilitation of fish stocks, principally lake trout, could not begin until the sea lamprey was brought under control. A special agency, the Great Lakes Fishery Commission, was created in 1956 by a treaty between Canada and the United States to fund and coordinate existing efforts to control the sea lamprey. Initial control methods attempted to block spawning migrations into streams by means of mechanical and electrical barriers. This method proved ineffective. In 1957, after several years of research and testing thousands of chemicals, one was found which was toxic to the lamprey but not lethal to other fish. Treatment of lamprey-infested streams with the chemical 3-trifluoromethyl-4-nitrophenol (TFM) began in 1958. By 1962, 2 years after all known lamprey nursery streams had been treated in Lake Superior, success was verified when the number of adult lampreys at assessment barriers was reduced nearly 85% (Baldwin, 1964). The incidence of lamprey wounds on lake trout dropped sharply, and survival of lake trout increased dramatically in Lake Superior. A method of control had been found just in time to save the last natural population of lake trout in the Great Lakes. The first complete treatment of all Lake Michigan lamprey-infested streams was completed in 1963, Lake Huron in 1970, and Lake Ontario in 1972. Chemical treatment of streams at intervals of 2-4 years must continue, however, if the rehabilitation of lake trout and other species is to become permanent.

The introduction of Pacific salmon in the Great Lakes had been attempted many times, but had produced limited results until the successful introduction of the coho salmon (Oncorhynchus kisutch) in Lake Michigan in 1966. By 1969 coho and chinook salmon (Oncorhynchus tshawytscha) had been introduced into all the upper lakes. The purpose of stocking coho and chinook salmon in the Great Lakes was to increase the sport fishing potential and not to establish self-sustaining populations. "Successful" introduction, therefore, relates to rapid growth and high survival rates. The pink salmon (Oncorhynchus gorbuscha), however, was an "unplanned" plant in Lake Superior, where it succeeded in establishing spawning runs in 1959 and by 1975 had become established in Lakes Huron and Michigan.

Supplemental plantings of lake trout, following lamprey control, have been made since 1958 in Lake Superior. The stocks have been built up to near pre-lamprey levels. Reproduction of hatchery-reared fish has been disappointing, however. Only in the last 2 or 3 years has the outlook improved, when increasing numbers of young native trout have been reported.

The reintroduction of lake trout in Lake Michigan, beginning in 1965, has proved extremely successful in terms of survival and growth. No evidence of reproduction, however, has been reported. Lake trout are now being stocked in Lakes Huron and Ontario. Biologists continue to be optimistic about the reestablishment of self-sustaining populations of lake trout in all the Great Lakes, except Erie.

Salmonids other than lake trout and Pacific salmon have been stocked in the Great Lakes since the lamprey has been controlled. Steelhead trout (rainbow trout), brown trout, brook trout, and Atlantic salmon are now stocked in the lakes. Over 20 million salmonids annually are stocked in the Great Lakes. In 1974 the first experimental plant of hatchery-reared saugers was stocked in Lake Erie.

Environmental Changes, 1950-1975

Scientific investigations of environmental conditions of the Great Lakes have increased exponentially during the past 25 years. Changes in fish populations, benthic organisms, plankton, and water quality are now measured with greatly improved accuracy and frequency. The ability to control environmental conditions and to understand ecological interactions, however, remains a goal of the future.

Chemical changes--The increase in major ions continued in all the lakes except Superior during the last 25 years. The rates of increase in all the ions except calcium remain high for Lakes Ontario and Erie (Table 9). Population increases also were substantial in the basins of Lakes Ontario and Erie (Table 3) and probably account for the chemical changes.

TABLE 9. ESTIMATED AVERAGE CONCENTRATIONS OF DISSOLVED CHEMICAL CONSTITUENTS IN THE GREAT LAKES IN 1970 (EXPRESSED IN MG/LITER)[a] WITH PERCENTAGE CHANGE SINCE 1950 IN PARENTHESES

Lake	Total dissolved solids	Calcium	Sulphate	Chloride	Sodium and potassium
Superior	55 (-2)	13 (0)	4 (0)	2 (0)	2 (-33)
Michigan	155 (3)	34 (0)	20 (43)	7 (40)	5 (0)
Huron	115 (3)	27 (12)	17 (31)	7 (17)	4 (0)
Erie	206 (21)	38 (0)	27 (17)	27 (42)	14 (56)
Ontario	210 (22)	40 (5)	30 (20)	29 (53)	15 (50)

[a] From Weiler and Chawla, 1969.

Critically low dissolved oxygen (DO) concentrations had not been reported in the open waters of the Great Lakes until 1953. In that year Britt (1955) measured DO concentrations less than 1 mg/liter in the western basin of Lake Erie. Although the low DO levels lasted only a few days, it caused a substantial mortality in the burrowing mayfly (Hexagenia) population. In some areas of the western basin the entire population was killed, where more than 1,000 mayfly nymphs per square meter had previously been found (Britt, 1955). The first extensive zone of low DO (less than 1 ppm) was measured in 1959, in the western portion of the central basin of Lake Erie. An interagency synoptic survey of this basin in 1959 found an area of approximately 1,400 square miles which contained less than 1 ppm of DO in the hypolimnion. These conditions of low DO undoubtedly had occurred before 1959 (Carr, 1962).

Dissolved oxygen levels of less than 1 mg/liter occur annually in the bottom waters of the central basin of Lake Erie and by 1974 covered several thousand square miles. Oxygen depletion also has been reported in southern

Green Bay (Lake Michigan) and the Bay of Quinte (Lake Ontario). Low DO levels in the open waters of the other lakes have not been reported. The virtual extinction of the sauger and blue pike and the decline of the walleye population in Lake Erie are thought to be partially caused by the low DO conditions (Smith, 1974).

Toxic Substances in Fish

Chemical contaminants in Great Lakes fish have been measured with increasing frequency in the past decade (1965-75). Measurements were first made in 1965 of the residues of the insecticides DDT and dieldrin in Great Lakes fish. All 28 species for which DDT and dieldrin analysis has been made contained measurable levels. Several species (chubs, lake trout, lake herring) from Lake Michigan exceeded the U.S. Food and Drug Administration's (FDA) tolerance level of 5 μg/g in fish used for human consumption (Reinert, 1970). Since the use of DDT was banned in 1972, the level in Lake Michigan fish has decreased rapidly, from an average of 10 μg/g in bloater chubs before 1972 to less than 3 μg/g in 1974.

During the same period in which DDT levels were decreasing in Great Lakes fish, polychlorinated biphenol (PCB) levels were increasing. Again, the species containing the highest levels were lake trout and bloater chubs in Lake Michigan. The average concentration of PCB in Lake Michigan lake trout above 24 inches exceeded 20 μg/g in 1974. Concentrations above the FDA's 5 μg/g tolerance level have been reported in fish from Lake Ontario and Lake Huron, as well as Lake Michigan.

In 1969 mercury levels in excess of the FDA's tolerance level of 0.5 μg/g were discovered in several species of fish (including walleye and white bass) from Lakes St. Clair and Erie. Mercury levels above 0.5 μg/g were also reported from Lakes Superior and Ontario. Two years following curtailment of the source of mercury pollution to Lake St. Clair, the levels in fish began to decrease. In two instances (DDT and mercury) stopping the sources of chemical contaminants resulted in the rapid decline of the toxicants in the environment. This success should give support to continued efforts to solve problems by eliminating the direct cause.

The contamination of Great Lakes fish with levels of DDT, PCB, and mercury exceeding the FDA tolerance level has resulted in great financial hardship to the commercial fishing industry. Direct or even indirect adverse effects on the fish populations of the Great Lakes have not been detected. Apparently, DDT and PCB in the low nannogram-per-liter levels in the open lake waters have, through biomagnification, reached the microgram-per-gram level in fish tissue.

Changes in Benthos and Plankton

Changes in bottom-dwelling organisms have been documented by several investigators within the past 20 years: Britt (1955) and Carr and Hiltunen (1965) for Lake Erie; Schneider, Hooper, and Beeton (1969) for Saginaw Bay; Hiltunen (1967) for Lake Michigan. In all of these studies the changes have been from the more "pollution-intolerant" organisms (mayflies, caddisflies, amphipods) to "pollution-tolerant" forms (primarily oligochaetes and midge larvae) and from greater to lesser species diversity. The geographic areas affected by loss of intolerant organisms are being extended further into the lakes from the pollution sources (primarily river mouths). The effects of these changes on fish populations will remain speculative until the interactions can be quantitatively assessed.

Phytoplankton and zooplankton populations have also changed markedly in many areas of the Great Lakes (Beeton, 1969). The changes in phytoplankton have been from dominance by multispecies diatom communities to species of green and blue-green algae more tolerant of eutrophic conditions. Zooplankton communities have reacted similarly (Beeton, 1969), resulting in a loss of species diversity and increases in species associated with eutrophic environments. Again, the relation of these changes in fish populations is incompletely understood.

CONCLUSIONS

It is obvious that the environment in many areas of the Great Lakes has deteriorated. Assigning direct cause and effect to changes in specific populations or species of Great Lakes fish is difficult and controversial. Heavy exploitation of many stocks is undoubtedly a factor in the decline of many species. Changes in water chemistry, plankton, bottom fauna, and unexploited fish species, however, clearly show that factors other than fishing have drastically changed the characteristics of the Great Lakes.

Now that we know our capabilities, how can we avoid past mistakes and stop, or perhaps even reverse, the trend toward environmental chaos? One possibility is to understand the forces that operated in the past to produce present conditions. Scientists and administrators with responsibility for protecting the aquatic environment can learn much from the perturbations foisted on the Great Lakes. For example, early recognition of the effects of unmanaged commercial fishing could have prevented, or at least delayed, the decimation of many fish populations. Wise management of the uses of tributary streams would have saved many stocks of anadromous species. It is difficult, however, to blame these errors of omission on our predecessors, for they did not have the advantage of hindsight to improve their foresight. Our generation has no such excuse. Opportunities missed in the past to protect the aquatic communities are gone, but opportunities remain to save and rehabilitate our aquatic environment.

Recognition of environmental degradation in the Great Lakes has led Canada and the United States to a firm commitment to halt and reverse this trend. Evidence of success in this endeavor is already apparent. Rehabilitation of many tributaries has permitted the establishment of spawning runs by anadromous species. Levels of DDT in fish tissue have decreased as much as 80% after the use of the insecticide was banned. More comprehensive and better treatment of municipal and industrial waste has resulted in noticeable improvements in the quality of receiving waters.

Let us hope that by the year 2000 the history of changes in fish species of the Great Lakes will show only increases in native species between 1975 and the new century.

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SECTION 19

SEA LAMPREY (PETROMYZON MARINUS LINNAEUS) IN THE SAINT LAWRENCE GREAT LAKES OF NORTH AMERICA: EFFECTS, CONTROL, RESULTS

Carlos M. Fetterolf, Jr.

The sea lamprey (Petromyzon marinus Linnaeus 1758) is in the class Agnatha, subclass Cyclostomata (Marsipobranchii), order Petromyzontiforms (Hyperoartia), and family Petromyzontidae. It is anadromous over most of its range (Figure 1), spending its parasitic adult life in the sea, but is landlocked in the Great Lakes and a few lakes in New York State.

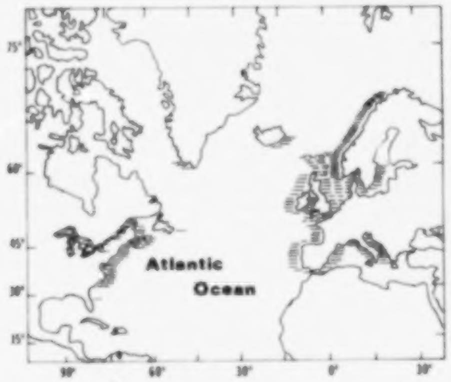


Figure 1. Range of the sea lamprey. Modified from Leim and Scott (1966).

Sea lampreys ascend freshwater tributaries and prefer a stony, gravelly bottom for spawning (Figure 2). Adults excavate depressions about 15 cm deep and 0.6-1 m in diameter in which the landlocked Great Lakes females deposit about 50,000 - 70,000 eggs each. Adults die after spawning. Eggs hatch into larvae (ammocetes), blind and toothless with a flexible, fleshy hood overhanging the mouth. Ammocetes live 3-14 years as filter feeders in burrows constructed in soft sediments of tributaries. Transformation (metamorphosis) involves disappearance of the hood and development of teeth on the tongue and a buccal funnel with teeth radiating in all directions from the mouth (Figure 3). Spawning Great Lakes sea lampreys are 30-60 cm long. Ammocetes reach about 12-16 cm in length before transformation.

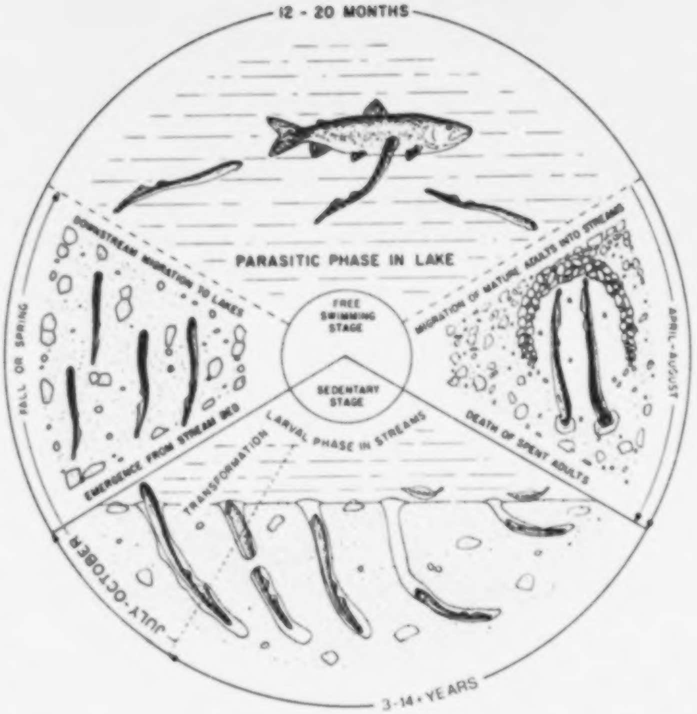

12 - 20 MONTHS
PARASITIC PHASE IN LAKE
DOWNSTREAM MIGRATION TO LAKES
MIGRATION OF MATURE ADULTS INTO STREAMS
FREE SWIMMING STAGE
SEDENTARY STAGE
FALL OR SPRING
APRIL-AUGUST
EMERGENCE FROM STREAM BED
DEATH OF SPENT ADULTS
LARVAL PHASE IN STREAMS
TRANSFORMATION
JULY-OCTOBER
3-14+YEARS

Figure 2. Life cycle of the sea lamprey (from Crowe, 1975).

"Transformers" (metamorphosed larvae) move downstream to lake or sea where they feed by attaching themselves to fishes, rasping a hole through the body covering and sucking body juices. Depending in part on hunger and size of the adult lampreys and size of their prey, one feeding can be fatal or the prey can withstand several attacks.

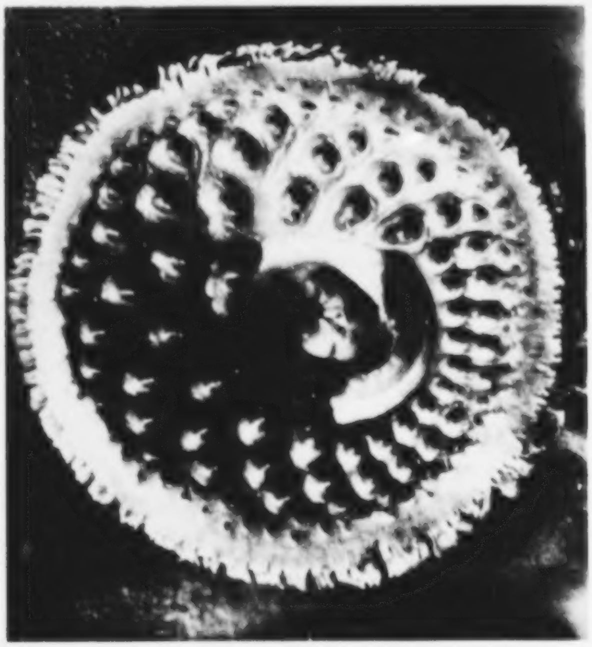


Figure 3. The mouth of the sea lamprey, which is lined with horny teeth surrounding a rasping tongue in the center.

Passage of the sea lamprey to the upper Great Lakes was blocked by Niagara Falls (about 50 m high) between Lakes Ontario and Erie. Completion of the Welland Canal for shipping in 1829 enabled the sea lamprey to bypass Niagara Falls. Moving up the Great Lakes, the lamprey was recorded in Lake Erie in 1921. The mid-1930's the animal had reached Lakes Huron and Michigan and by the 1940's it was firmly established in Lake Superior. By the mid-1940's fish stocks in Lakes Huron and Michigan had been severely damaged, and similar damage was predicted correctly for Lake Superior. The catastrophic decline in lake trout (Salvelinus namaycush) in relation to sea lamprey invasion is best traced in Lake Superior (Figure 4).

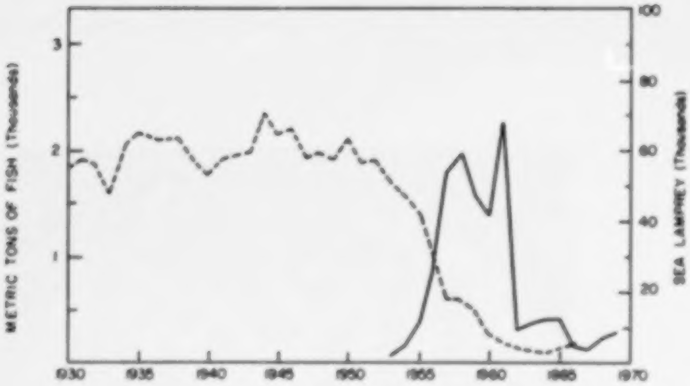
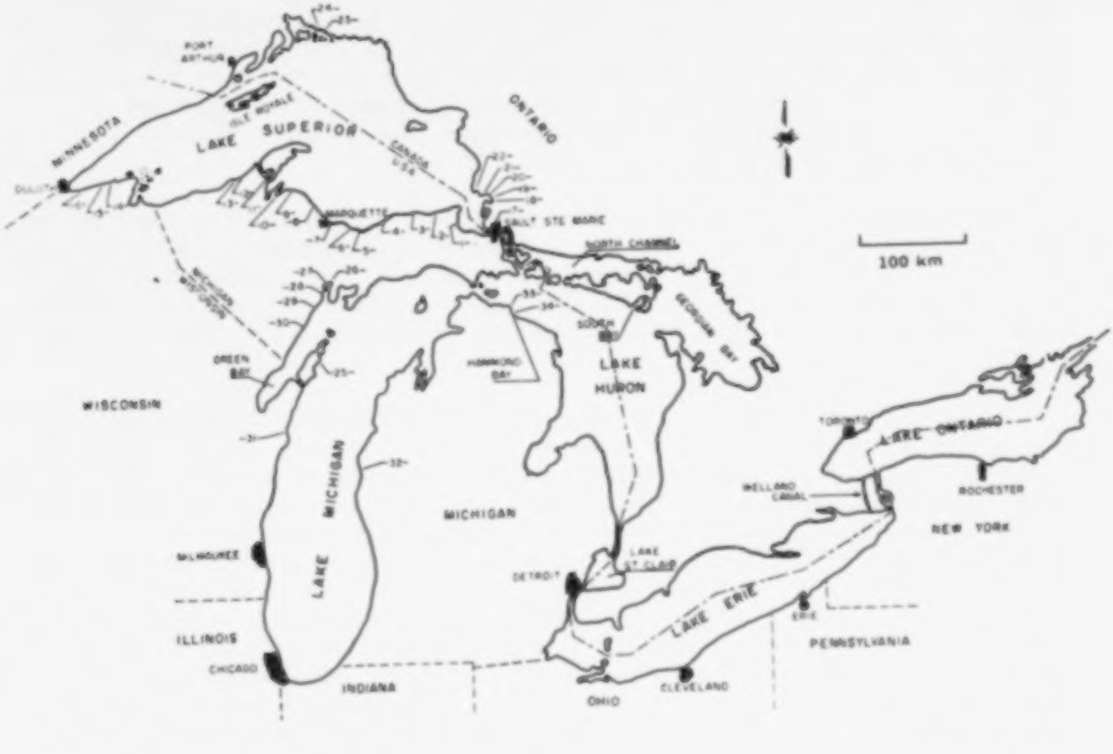


Figure 4. Production metric tons) of lake trout, 1930-66 (broken line), and number of sea lampreys caught in index streams in Lake Superior, 1953-69 (solid line). From Smith, 1971.

Fisheries agencies recognized the urgent need for a control program, and because the Great Lakes are bordered by two countries, United States and Canada, including one Canadian province and eight U.S. states, the need for international and interstate cooperation was imperative (Figure 5). In 1948 a committee representing these jurisdictions was established. Initial control efforts were experimental and uncoordinated because so many agencies were involved, and funding was not assured. In 1955 a Convention on Great Lakes Fisheries was ratified by the United States and Canada which established the Great Lakes Fishery Commission. The commission's program is divided into two major segments: (1) sea lamprey control and (2) coordination of fisheries research and management. The commission has no regulatory authority, but provides the forum in which mutually beneficial courses of action are developed. Funding is through the Department of External Affairs in Canada and by legislative appropriation to the Department of State in the United States. The early programs of the study of sea lamprey life history and distribution, development and testing of barriers in streams, and screening of chemicals that would selectively destroy larvae were continued, coordinated, refined, and expanded under commission auspices after 1955. The control programs are carried out by agents of the commission, the U.S. Fish and Wildlife Service, and the Canadian Department of the Environment. Research is funded by, and has been done mostly by, the U.S. Fish and Wildlife Service.

Sea lampreys are most vulnerable to current control methods when concentrated in streams as adults on upstream migrations, as larvae in the streams, or as transformers moving downstream. While tests were carried on to find a selective chemical, a control program by means of mechanical and electromechanical barriers was operated. At its peak in 1959 the program included about 135 barriers in the United States and Canada, but 401 of the 5,747 tributaries to the Great Lakes are known to produce sea lamprey. The effectiveness of barriers as a control method was never adequately determined, but there is no doubt that barriers are effective in killing large numbers of adult sea lampreys. The barrier program was discontinued as the major control method after the discovery of a selective chemical. Electrical barriers are still used at selected sites as a means of assessing program effectiveness and to provide control over experimental areas.


PORT ARTHUR
ISLE ROYALE
MINNESOTA
LAKE SUPERIOR
ONTARIO
CANADA
USA
DULUTH
MARQUETTE
SAULT STE MARIE
NORTH CHANNEL
100 km
MICHIGAN WISCONSIN
GEORGIAN BAY
SOUTH
LAKE HURON
HAMMOND BAY
GREEN BAY
WISCONSIN
LAKE MICHIGAN
MICHIGAN
TORONTO
LAKE ONTARIO
WELLAND CANAL
ROCHESTER
NEW YORK
LAKE ST CLAIR
DETROIT
MILWAUKEE
ILLINOIS
CHICAGO
INDIANA
OHIO
CLEVELAND
LAKE ERIE
ERIE
PENNSYLVANIA
24
23
22
21
20
19
18
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33
34

Figure 5. The Great Lakes. Numbers indicate streams on which sea lamprey counting weirs were installed. From Smith, 1971.

Some 6,000 chemicals were screened through laboratory bioassay over a 7-year period (Applegate et al., 1957). Promising toxicants were field tested in 1957 and 1958. These successful tests led the commission in 1958 to adopt use of two chemicals, 3-trifluoromethyl-4-nitrophenol (TFM) and 2', 5-dichloro-4'nitrosalicylanilide (Bayer 73), as the major sea lamprey control technique. Routine stream treatments are carried out with TFM or with TFM plus a small amount (1-4%) of powdered Bayer 73. The addition of Bayer 73 reduces up to one-half the amount of TFM required and greatly reduces the cost of treatments. A granular form of Bayer 73, which settles to the bottom before chemical release, is also used in difficult-to-reach areas during treatment with TFM, but is more frequently used as a collecting tool in surveys. At proper concentrations the chemicals destroy sea lamprey larvae without significantly affecting other fauna and flora.

A defined range of concentrations, dependent on alkalinity, pH, and temperature, must be maintained for several hours throughout the treatment area. Field bioassays, conducted in mobile units, identify the lowest concentration of TFM that kills 100% of sea lamprey larvae in 9 hr or less and the highest concentration that does not kill more than 25% of the test species (usually rainbow trout, Salmo gairdnerii) in 18-24 hr (Kanayama, 1963). These criteria provide safety factors at both extremes. Stream concentrations are maintained between these limits by controlled applications at several stations on the stream. The defined range can vary between 1.0 and 2.3 mg/liter toxicant on low alkalinity streams (10-20 mg/liter as $CaCO_3$) and between 6.7 and 17.0 mg/liter toxicant on high alkalinity streams (163 mg/liter as $CaCO_3$).

Sea lamprey control with lampricides was initiated in Lake Superior in 1958 and expanded to Lake Michigan in 1960, to Lake Huron in 1966, and Lake Ontario in 1971. The first "round"[1] of treatments was completed in Lake Superior in 1961; in Lake Michigan in 1966; in Lake Huron in 1970; and in Lake Ontario in 1972. The total number of treatments since 1958 exceeds 1,000.

In Lake Superior, where the control program has been in operation for the greatest number of years and where its effectiveness has been most carefully evaluated, sea lamprey abundance has been reduced by about 90%. A quantitative measure of sea lamprey abundance has been obtained from counts of mature (spawning run) sea lampreys reaching assessment barriers. Numbers of mature sea lampreys in the Lake Superior spawning runs declined sharply in 1962, the year after the first round of stream treatments had been completed (Figure 6). The decrease was accompanied by a marked decline in the incidence of fresh sea lamprey wounds on lake trout and later by an improved survival of lake trout to older age and larger size. Equivalent quantitative data are not available for Lakes Huron and Michigan, but the responses of sea lamprey and fish populations to control efforts have been similar to those in Lake Superior.

The Great Lakes Fishery Commission is concerned that the control program is singularly dependent on chemicals, primarily TFM. Only one chemical manufacturer submits bids. Costs have risen sharply to $13.18 kg, and we use over 45,360 kg a year. Early in the program it was necessary to treat each stream only once every 4 years. However, the average ammocete is now transforming in a shorter period, presumably because of reduced competition for food and space. This requires more thorough surveys and more frequent treatments and emphasizes the need for alternative controls. The commission is developing an integrated control program including permanent barriers on selected streams and is sponsoring research into chemical attractants and repellants as well as chemosterilants.

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[1] A "round" denotes that all known sea-lamprey-producing streams tributary to that lake have received one chemical treatment.

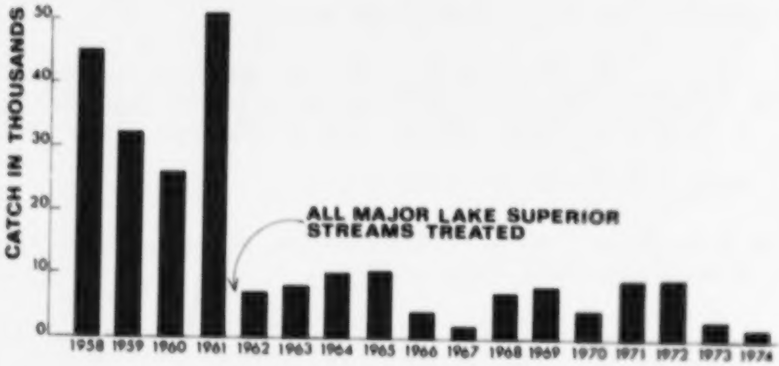

Figure 6. Sea lamprey catch from eight streams tributary to Lake Superior. Modified from Crowe, 1975.

In 1971 comprehensive studies of the immediate and long-term effects of lampricides in the environment were initiated. Results suggest that the effects are very small, that the chemical control program can continue, and that registration of the lampricides by the Environmental Protection Agency will be forthcoming upon completion of the required studies. About $1.2 million were allocated to do this registration-oriented research in 1971-74.

The annual budget of the commission is about $4 million. Without control of sea lamprey the sport and commercial fisheries would be limited. To date, with fish stocks only in the process of rehabilitation, the value of the Great Lakes sport fishery is estimated at over $350 million. The commercial fishery is valued at $19 million at the dock and approaches $100 million at the market. There is an excellent return from the money invested in sea lamprey control.

ACKNOWLEDGEMENTS

I have been associated with the Great Lakes Fishery Commission since July 1975. As a recent executive secretary I take no credit for the progress and programs reported above. The credit is deserved by the pioneer sea lamprey control workers in the U.S. Fish and Wildlife Service, the Canadian Department of the Environment, the Great Lakes Fishery Commission, all cooperators, and the administrations that supported them.

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VOLUME II

PROCEEDINGS OF THE SECOND USA-USSR SYMPOSIA ON THE EFFECTS OF POLLUTANTS UPON AQUATIC ECOSYSTEMS

June 22-26, 1976
Borok, Jaroslavl Oblast
USSR

Edited by

Wayland R. Swain
and
Nina K. Ivanikiw

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

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FOREWORD TO VOLUME II

These Proceedings result from the second symposium held by Project 02.02-1.3 of the US-USSR Joint Agreement in the Field of Environmental Protection, established in May, 1972.

Both broad review and narrowly specific papers were presented by participants from both countries in an effort to continue the joint procedural, technological and methodological exchange and familiarization begun at the Duluth Symposium in October of 1975. Learning does not occur and subsequent understanding and application must be based on a foundation of fact. The atmosphere of mutual interest, candor and respect which surrounded this symposium enabled another series of steps in the learning process. Perhaps the philosophy underlying this symposium, and the project itself is best expressed by an old saying, which transliterated from the Russian approximates: Vyek zhee-vee, Vyek oo-chee, Live a lifetime, learn a lifetime.

PREFACE TO VOLUME II

This volume contains fifteen papers presented at the Second US-USSR Symposium on the Effects of Pollutants on Aquatic Ecosystems. All of the papers were presented in English or Russian with simultaneous translations into the corresponding language at Borok, Jaroslavl Oblast, USSR during June 22-26, 1976 at the Institute for the Biology of Inland Waters of the USSR Academy of Sciences.

Professor N.V. Butorin, Director of the Institute and Project Leader for the Soviet side, served as official host for the American delegation and has assumed the responsibility for the publication of these proceedings in the Russian language. This joint bilingual publication represents a reaffirmation of the continuing commitment pledged by both countries to cooperative environmental activities.

INTRODUCTION

The Joint US-USSR Agreement on Cooperation in the Field of Environmental Protection was established in May of 1972. These proceedings result from one of the projects, Project 0.2.02-1.3, Effects of Pollutants Upon Aquatic Ecosystems and Permissible Levels of Pollution.

As knowledge related to fate and transport of pollutants has grown, it has become increasingly apparent that local and even national approaches to solving pollution problems are insufficient. Not only are the problems themselves frequently international, but an understanding of attentive methodological approaches to the problem can avoid needless duplication of efforts. This expansion of interest from local and national represents a logical and natural maturation from the provincial to a global concern for the environment.

In general, mankind is faced with very similar environmental problems regardless of the national or political boundaries which we have erected. While the problems may vary slightly in type or degree, the fundamental and underlying factors are remarkably similar. It is not surprising, therefore, that the interests and concerns of environmental scientists the world over are also quite similar. In this larger sense, we are our brother's brother, and have the ability to understand our fellowman and his dilemma, if we but take the trouble to do so. It is this singular idea of concerned scientists exchanging views with colleagues that provides the basic strength for this project. While our methods may vary, our goals are identical, and therein lies the value of such a cooperative effort.

Wayland R. Swain, Ph.D.
Project Officer, U.S. Side

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ACKNOWLEDGEMENTS

In any project of the scope and complexity of this effort, the Project Officers become increasingly indebted to a large number of individuals who contribute their time and effort with no thought of personal gain. Unfortunately, the list of persons who materially aided the effort is too extensive to allow a complete discussion. However, while those persons who made outstanding contributions to the success of this project are acknowledged below, the editors also wish to thank all those others, both Soviet and American, whose efforts and assistance smoothed the way to a satisfactory completion of this phase of the project.

Sincere thanks are extended to Ms. Elaine Fitzback and Mr. Igor Kozak whose assistance with the translations have made possible the publication of these proceedings. The substantial contributions and tireless efforts of Ms. Virginia Shannon, Ms. Debbie Caudill, and Ms. Dawn Armatis to the preparation of the proceedings are acknowledged with deep appreciation.

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SECTION 1

TOXICITY TESTS IN THE REGULATION OF WASTE DISCHARGES IN THE UNITED STATES

Peter Doudoroff

I have recently undertaken a cursory examination of much of the Soviet literature in the field of aquatic toxicology. I have found in that literature no evidence to indicate that biological tests for toxicity of wastewaters are often required or routinely performed in regulating waste discharges in the U.S.S.R. Neither the standardization of toxicity bioassay methods, nor the formulation or systemization of procedures for the application of bioassay results in the control of waste disposal seems to have received nearly as much attention in the U.S.S.R. as in the United States. Some of the pertinent ideas and current practices of American workers that are briefly and incompletely reviewed here, and my own thoughts concerning their relative merits, may be interesting and useful, therefore, to those Soviet scientists who must deal with regulatory problems. I know that many industrial effluents and their individual components have been tested by Soviet investigators for acute and chronic toxicity to fish and other aquatic organisms. But it is not that scientific research into the toxicity of these various water pollutants to which I refer. I am speaking of regular biological testing of wastewaters by technicians to verify compliance with, or to detect violations of, some specific regulatory requirements limiting the discharge of toxic wastes.

Toxicology studies reported in the Soviet literature often have been directed toward the determination of maximum acceptable concentrations of paricular toxic substances to actual concentrations of which can be determined by chemical analysis is receiving waters. In the United States, also, much research of this kind has been done and continues. Deficiencies in the chemical criteria of water or wastewater quality so developed, and a need for more reliance on biological tests of effluents in pollution control have long been apparent to many American workers. We realized that many of the toxic components of industrial wastes had not been, and would not soon be identified, or could not be reliably measured for lack of suitable analytical methods. Further, the toxicities of even identified and measurable compounds to important species of fish (and the interactions of these toxicants with natural components of the various receiving waters and among themselves in complex mixtures) were mostly unknown and unpredictable. Largely for these reasons, my colleagues and I long ago undertook the standardization of toxicity bioassay methods suitable for routine application in industrial laboratories, using locally important fish as test animals and waters receiving the tested wastes as diluents (Hart, Doudoroff, and Greenbank, 1945; Doudoroff, *et al.*, 1951). The methods developed and recommended have been widely adopted by other investigators, and by regulatory agencies and industrial organizations in the United States and elsewhere. They have appeared repeatedly, with some minor modifications or refinements, in manuals of currently approved, standard practice, such as the eleventh edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association *et al.*, 1960) and the subsequent editions. Similar methods for evaluation of the toxicity of water pollutants to organisms other than fish have been developed, but their use in the United States outside of research laboratories has not yet been extensive.

Chemical criteria of water quality can be very useful, and cannot be entirely ignored in controlling pollution for the protection of aquatic life. However, deficiencies in this area, even after the great technological advances of recent years, are still very real and apparent. Thus, frequent reliance on toxicity bioassays of effluents regulating the discharge of toxic wastes is still necessary. Several ways in which these toxicity tests can be used in controlling water pollution have been proposed by biologists and tried by regulatory agencies.

The maximum safe or harmless concentration of an industrial waste or other toxicant in a receiving water cannot be directly determined, by performing a toxicity test of short duration, e.g., a 96 hour test. Much longer and more difficult tests can be successfully undertaken only in research laboratories, and cannot be frequently repeated. When only the acute toxicity of an effluent is known, its highest permissible concentration in the receiving water must be computed by some prescribed formula that has been judged to be appropriate. For example, the concentration found by experiment to be fatal in 48 hours to just 50 percent of test animals, termed the 48-hour "median tolerance limit" or "median lethal concentration" (LC_{50}), may be simply multiplied by a fractional "application factor", e.g., 0.10, to obtain the permissible or presumably safe concentration. This formula, with the application factor of 0.10, was first recommended tentatively by the Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1955) as one believed to be sufficiently but not unreasonably restrictive. That committee noted, however, that smaller or larger application factors may be often fully justifiable. The recent trend has been to reduce the permissible concentrations, substituting the 96-hour LC_{50} for the 48-hour value, and using smaller application factors in the formula. More complicated formulas have been proposed (Hart, Doudoroff, and Greenbank, 1945; Doudoroff *et al.*, 1951; footnote No. 7). For some time they attracted much favorable attention, but regrettably they have not proved sufficiently useful for wide-spread adoption by regulatory agencies.

For each of a large variety of toxic substances, an individual application factor has been recently proposed (National Technical Advisory Committee on Water Quality Criteria, 1968; Committee on Water Quality Criteria, National Academy of Sciences and National Academy of Engineering, 1972/1973). The recommended values are based on the results of laboratory studies in which median lethal concentrations of the toxicants were related to the highest levels that were apparently harmless to the test animals in experiments of long duration. The usefulness of these widely ranging application factors in dealing with industrial wastes that are complex mixtures of variable, and often incompletely known composition, is questionable. Application factors most appropriate to different kinds of industrial wastewaters differ greatly. Prescribed application factors also vary with the value of commercial or recreational fisheries that are to be protected, some of which merit a high degree or level of protection than others. Economic and social considerations cannot be overlooked in deciding how much risk of impairment of fish production by waste discharges is to be deemed acceptable (Warren, 1971, pp. 15-23, 375-386).

After the test animal to be used, a suitable test temperature and exposure period, and an appropriate application factor has been carefully chosen, an announced enforcement of this regulation next must be undertaken. To determine whether or not the concentration of an acutely toxic effluent that has been judged permissible in a receiving body of water is outside an allowable dispersion or mixing zone, the amount of dilution of the effluent within the mixing zone must be known. Only a concentration of the effluent (percent by volume) equal to the product of its concentration in the receiving water (at the boundary of the mixing zone) and the reciprocal of the prescribed application factor needs to be tested for toxicity. If, at any time, this concentration is found to kill more than 50 percent of the test animals in the specified exposure period, either the toxicity of the effluent or the rate of its discharge may be regarded as excessive. If 50 percent or more of the test animals survive in such tests, the permissible concentration is not exceeded, and hence, the regulatory requirement is not being violated.

The above procedure is applicable to all effluents that are toxic enough to kill at least half of the test animals in the prescribed test period when they are not diluted. If an effluent is of low toxicity, but the product of its concentration (percent by volume) and the reciprocal of the prescribed application factor is greater than 100 percent, one cannot reasonably conclude from the result of the acute toxicity test that aquatic organisms are not being endangered. The possibility of serious chronic or sublethal toxicity of the effluent cannot be judged negligible in the absence of better evidence. Tests for toxicity of such effluents that have no readily measurable acute toxicity must be required to protect aquatic life adequately.

Reliance on acute toxicity tests alone in regulating discharges of any toxic wastes can occasionally lead to serious error. The regulatory practices considered above are based on certain assumptions which certainly cannot be always valid. The chronic or sublethal toxicity of a complex industrial waste at low concentrations can be quite independent of its acute toxicity at much higher concentrations, since the causative agents of these variations in toxicity can be entirely different components of the wastes. Further, environmental variables, such as natural water quality and temperature, can influence toxicity in very different ways, even when the same toxicant is the active agent at low and high concentrations. A species of fish that is more resistant than another to the lethal action of a toxicant can be more susceptible than the other to sublethal injury by the same compound.

The value of acute toxicity tests in the assessment and control of pollution can be often reasonably questioned. For entirely different reasons, chemical data may also be very misleading. Until analytical methods are perfected, and the problems of interpretation of the chemical information are solved, continued heavy reliance on toxicity tests of short duration appears to be warranted.

For some purposes, the acute toxicity test is clearly the best possible test. Even when dilution of toxic effluents is sufficient to prevent damage to aquatic life outside the mixing zone, fish may be killed when they enter the mixing zones if the dilution is not very rapid. To avoid fish kills in the immediate vicinity of wastewater outfalls, it may be necessary to limit the toxicity of the effluent without regard to the amount of further dilution. It is evident that the toxicity must be sufficiently low so that the exposed fish will be overcome rapidly by the effluent, unless they are known to be attracted by the effluent or likely to remain in it for long periods for other reasons. The safe level of acute toxicity varies with the area of the mixing zone as well as the ability of fish to avoid high effluent concentrations. Acute toxicity tests and limits are most appropriate for the regulation of some infrequent discharges of toxic wastes that are of sufficiently short duration, that there is no need to protect organisms against chronic toxicity of the wastes. The toxicity of intermittantly discharged wastewater can be relatively high without causing serious damage, but the amounts or rates of their dilution, and the duration of the discharges must be considered prior to setting limits.

Some regulatory authorities have favored much more stringent and uniform toxicity limits independent of the amounts of dilution of the effluents, the frequency, and the duration of the discharges. For example, 96-hour survival has been required of an average of at least 90 percent, or even of no less than 80 or 90 percent at any given time (in every test), of prescribed test animals (fish) in undiluted effluents of various kinds. Specifically, application has been made to pulp and paper mill effluents diluted to 65 percent of their full strength, without regard to plant location. Various arguments have been advanced in support of such uniform requirements unrelated to the assimilative capacities of waters receiving the wastes. The enforcement of this type of regulation is simplier than the limitation of toxic waste concentrations in the receiving waters, and some people believe that this solution more equitable than the latter restrictions. However, when the dilution factor related to wastewaters is great, the requirements in question can be quite unnecessarily restrictive, necessitating costly waste treatment or other expensive measures that result in no real benefits. On the other hand, when the dilution is slight, the same requirements can be quite inadequate for protection of aquatic life against chronic or sublethal toxicity of the wastes.

For the above reasons and others beyond the scope of this paper, I strongly disapprove of such pollution-control measures or requirements. They tend to discourage the selection of sites for industrial plants where effects on the environment will be minimized, because they do not permit reduction of waste disposal costs by choosing the more favorable locations. They also discourage the conservation of water by industry, because more frugal use of water that results in great reductions in volume of effluents, usually results also in some increases of the concentrations of toxicants in the effluents.

Instead of arbitrarily limiting the concentrations of toxicants in effluents for protection of aquatic life outside the mixing zones, it is surely more reasonable to limit the amounts of these harmful substances discharged per unit of time. These amounts can decrease while the toxicant concentration increase, if the volume of the effluents discharged per unit of time is simultaneously reduced. The measured toxicity of a wastewater is a function of the concentration of the toxicant or mixture of toxicants present. If all poisons were equally toxic, it would be a measure of their total concentration whenever several are present. The toxicity of any solution expressed in toxicity units (t.u.), sometimes called the "toxicity concentration". This is equal to the reciprocal of its median lethal concentration (LC_{50}) expressed as the decimal fraction by volume (percent by volume ÷ 100). For example, if the LC_{50} is 0.2 (or 20 percent) by volume, the toxicity is 1/0.2 or 5.0 t.u. An application factor prescribed for an effluent is equal numerically to its permissible concentration at the boundary of the mixing zone expressed in appropriate (corresponding) toxicity units. The expression of toxicity levels in such units has been shown by experiments to be useful in the estimation or prediction (by summation) of the toxicities of various mixtures of poisons whose individual toxicities have been determined (Brown, 1968; Warren, 1971, p. 210).

An approach to the regulatory problems that has recently been gaining favor in the United States and Canada is to express the output of toxicants from each important source as a value to which the name "toxicity emission rate" (T.E.R.) has been given (California State Water Resources Control Board, 1972). This value can be computed by dividing the rate of flow of the effluent by the determined median lethal concentration expressed as a decimal function by volume. For example if the determined 96-hour median tolerance limit or LC_{50} of an effluent is 0.20 (20 percent) by volume, and its flow rate is 2.0 m^3/min.:

$$\text{T.E.R.} = \frac{2}{0.2} = 10\ \frac{m^3}{\text{96-hr. } LC_{50} \cdot \text{min.}} = 10\ \frac{\text{t.u.} \cdot m^3}{\text{min.}},$$

where t.u. represents toxicity units based on results of 96-hour tests.

Uniform requirements that limit the T.E.R. per unit of industrial production of a given kind (for example, per ton of cellulose pulp produced per day by a sulfate-process pulp and paper mill) would be much more reasonable than are those that simply limit the toxicity of the effluents without regard to their discharge rate or volume. However, for reasons already noted, I cannot fully approve any waste-disposal regulations that are entirely independent of local conditions and needs, the manner of discharge of the wastes, and the ability of receiving waters to assimilate them without impairment of any beneficial uses of the waters. I believe that toxicity emission rates can best be limited so as always to permit reasonable utilization of the assimilative capacity of the receiving waters with no undue risk of injury to aquatic life by toxicants. the calculation and control of these rates may prove especially useful in protecting valuable organisms in waters that receive toxic wastes in considerable amounts from several sources.

Procedures for equitable apportionment of the assimilative capacities of waters for toxic pollutants among multiple sources of these wastes have not yet been thoroughly developed, and will not be discussed in detail here. Much attention recently has been given to this difficult regulatory problem in the United States, especially in California. Various proposals for its solution have been advanced and studied, but none, as yet, have been shown to be entirely sound nor widely accepted. When there are several important sources of toxic pollution of a water body, evaluation and consideration of the persistence, as well as the dilution, of the discharged toxicants in the receiving water may be necessary. The acute toxicity of combined, fresh effluents from different sources not separated by large distances can often be easily determined experimentally. Perhaps the combined toxicity can also be estimated with sufficient accuracy for regulatory purposes by measuring the toxicity of each effluent, and assuming an additive interaction of the poisons in the mixture. This assumption is implied by the proposed summation of toxicity emission rates. But when effluents from several plants are discharged into a body of water at points that are remote from each other, the nonpersistent toxicants from the different sources do not occur together in the receiving water. Unless most of the toxicants involved are very persistent, the safe discharge rates for the effluents can be much greater than they would be if all the effluents were discharged together at one point into a common mixing zone. The proper allowances to be made for natural self-purification of waters are not easily determined, and very little is known about the interaction of poisons at sublethal concentration levels. Measurements of rates of loss of toxicity under appropriate conditions in the laboratory can be useful in estimating levels of residual toxicity of polluted waters in nature. Without some such correction for natural self-purification, a value for the "toxicity concentration" (in t.u.) at a given point in a stream, computed by dividing the sum of the toxicity emission rates from all upstream sources by the stream flow (in m^3/min., for example), is not meaningful. But a correction for loss of toxicity can introduce an error in the opposite direction when the results of the calculation are applied in regulating the waste discharges. Because slowly acting, accumulative poisons tend to be also highly persistent, the appropriate application factor for the acute toxicity of a solution of nonpersistent and persistent poisons is likely to decrease as the solution ages.

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SECTION 2

TOXICOLOGICAL CONTROL OF POLLUTION OF FRESHWATERS

N.S. Stroganov

The methods of chemical, microbiological and sanitary/hydrobiological analysis have been well established for the evaluation of water quality. Each of the methods allows the characterization of water in any single aspect, i.e., chemical, epidemiological or sanitary significance.

At the present time, various toxic substances such as petroleum and its associated products, pesticides, heavy metals, detergents, metal organic compounds and many others are discharged into surface waters in ever growing amounts. The toxic substances impart an altered quality to the receiving waters, to which the aquatic organisms react with extreme sensitivity, but which is not evaluated by the methodology noted earlier. Thus, toxicology evaluations of water quality are required.

As industry develops, and as chemistry advances in various fields related to the economy, the increasing potential for pollution of the water requires the organization of toxicologic control of waste waters to assure continued quality of all large water-bodies and to provide more reliable protection for these surface waters. Using integrated models, toxicological control must characterize the quality of water in relation to the suitability for the life of aquatic organisms. it is expedient to exercise control of the toxicity of waters in two ways:

1. Evaluation of the toxicity of waste waters discharged from industrial sources, sewage plants, and points of population concentration, and

2. Evaluation of the toxicity and associated hazards of waters for aquatic organisms.

These varieties of control characterize both the quality of waters entering a water-body and the waters of the reservoir itself in which the self-purification has already begun or become pronounced. The essence of such toxicological control may be divided into two categories as follows:

The First Variant

Three organisms having varying degrees of sensitivity are suggested for testing for acute toxicity: waterfleas (*Daphnia magna*, Straus) as a sensitive organism; the large pond snail, *Limnea stagnalis* as an organism of intermediate sensitivity; and the guppy, *Lebistes reticulatus*, a fish of least sensitivity. The duration of the test is five days at a temperature of 17-22 °C. Survival and general condition (behavior) are the criteria of toxicity to the organism. Toxicity is estimated using a five degree system:

1. First Degree. Very acutely toxic. All organisms of the three species die within the first day.
2. Second Degree. Acutely toxic. All organisms of the organisms of the three species die within five days.
3. Third Degree. Toxic. From 70 to 100 percent of the *Daphnia* die; not more than 20 percent of the pond snails expire; and all guppies survive.
4. Fourth Degree. Slightly toxic. The mortality of *Daphnia* does not exceed 30 percent; and all of the pond snails and guppies survive.
5. Fifth Degree. Conventionally non-toxic. All of the organisms of the three species survive, and by outward appearance and behavior do not differ from control organisms.

When the variability in the degree of toxic waste water is considered, control of periodically diluted waste water is recommended. In this regard, waste water is diluted in the following order: 0 (initial), 5, 10, 100, 500 times with clean water from a river or brook containing no toxic substances. The scheme of the tests is identical to the initial waste water. The results obtained are expressed graphically by plotting the degree of dilution against the degree of toxicity. The resultant slope of the curve suggests either the rate of increase characterizing the toxicological danger of the waste water, or the rate of loss of the toxicant with dilution.

The first variety of control of toxicity may be used for differential determination of the degree of toxicity of waste waters discharged from different sources within a single industry. This enables the detection of the most dangerous sources and allows these waters to be directed to special treatment.

The Second Variant

The waters of large rivers, reservoirs and lakes do not possess the acute toxicity of waste waters, although they receive these waters. However, even in low concentrations, a prolonged influence of toxic substances on aquatic organisms leads to death or diminution of the numbers of the most sensitive of these organisms. These conditions transform individual aquatic communities and the ecosystem as a whole. Therefore, toxicological control is also necessary for the waters of rivers, reservoirs, and lakes which undergo anthropogenic influence.

The toxicological control of the second variety is assayed by pouring the water to be tested into 10 beakers, 100 ml in each. Clean water in the same quantity serves as a control. To each of the beakers one-two day old *Daphnia magna* are added. The duration of the experiment is 30 days at a temperature of 17-22 °C. The *Daphnia* are fed with *Chlorella sp.* and the water is changed every 3-4 days. The criteria of toxicity include survival, rate of reproduction (fecundity), time of maturation, and frequency of moulting.

The toxicity or harmfulness of the water for the organism is expressed by three degrees:

1. First Degree. Water is toxic. 50 percent or less of the *Daphnia* live less than 30 days.
2. Second Degree. Water is harmful. 80 percent or more of the *Daphnia* survive for 30 days, but fecundity is reduced by 25 percent or more as compared with control populations.
3. Third Degree. Water is clean. All the *Daphnia* survive, and the fecundity is not reduced by more than 25 percent in comparison with the control.

By simultaneously conducting toxicologic control of the first variety every 5-7 days while running the longer experiment of the second variety, the occurance of late results is minimized. Compensation is achieved by exerting simultaneous control of the discharge water and the receiving water of the water body. If periodic discharge occurs, detection is enabled by the toxicological control of the first variety.

Toxicological control does not indicate the nature of the toxicant, but does show the danger of the water in question for many aquatic organisms.

SECTION 3

TOXICITY OF EXPERIMENTAL FOREST INSECTICIDES TO FISH AND AQUATIC INVERTEBRATES

Richard A. Schoettger and Wilbur L. Mauck

INTRODUCTION

In 1952, the U.S. Forest Service began aerial spraying of insecticides to control outbreaks of the spruce breakdown (*Choristoneura fumiferana*). The first forest insecticide to be applied was the organochlorine compound, DDT, which was commonly applied at a rate of 1.12 kg per 9.3 liters per hectare (1 pound DDT per 1 U.S. gallon per acre). Applications of DDT continued for nearly 20 years before its further use was prohibited in 1969 by the U.S. Environmental Protection Agency. However, this compound was banned in the State of Maine for use as a forest insecticide as early as 1967.

DDT was of particular concern to environmental scientists because of its persistence, toxicity and bioaccumulation in non-target organisms. As early as 1963, DDT was recognized as an imminent hazard to terrestrial, avian, and aquatic fauna by the President's Science Advisory Committee. This committee recommended a reduction in the use of persistent pesticides. Certain uses of DDT were prohibited by the U.S. Department of Agriculture (USDA) in 1969 and an informal review of the remaining uses continued through 1970. On the basis of some 10,000 pages of testimony by more than 50 scientific experts, the Administrator of the Environmental Protection Agency issued an opinion and order, published July 7, 1972 in the Federal Register, cancelling or suspending all uses of DDT, except those related to human health (USDA, 1973).

After the banning of DDT, government officials and environmentalists urged the development and use of insecticides that were highly specific in their action, were more readily biodegradable, and did not bioaccumulate. Because research on radically new methods of control might consume excessive time or be unproductive, the U.S. Forest Service decided that investigations of DDT replacements should be directed only to chemicals already in production, or experimental compounds near the production stage (Schmiege *et al.*, 1970). Also, the compounds selected should be applied from the air, by spraying procedures that had been developed for DDT. Three conditions were to be met: (1) the insecticide should be more toxic to the western spruce budworm than to other organisms; (2) the insecticide and its degradation productions must not accumulate in plants or animals found in forest ecosystems; and (3) the insecticide spray should be directed efficiently to the target insect (Schmiege *et al.*, 1970).

Currently, the U.S. Forest Service is evaluating the efficacy of several organophosphate, carbamate insecticides, and insect growth regulators for the control of three major insect pests: the gypsy moth (*Porthetria dispar*), Douglas-fir tussock moth (*Hemerocampa pseudotsugata*), and spruce budworm. All three pests cause defoliation, but the spruce budworm has the most widespread distribution and causes the most damage.

The gypsy moth was introduced accidentally into North America in 1869 at Medford, Massachusetts, by a French naturalist who had intended to cross it with the silkworm (*Bombyx mori*). The spread of the gypsy moth from that source occurred in two ways: (1) by windblown dispersal of the newly hatched larvae, and (2) by inadvertent transport. The gypsy moth is now generally distributed in the northeastern and eastern portions of the U.S. It feeds primarily on leaves of hardwood trees (mainly oaks), but the late-instar larvae may also defoliate conifers (USDA, 1973b).

The Douglas-fir tussock moth is a native insect of western North America and has caused extensive damage to coniferous forests of Canada and the northwestern United States. It prefers to feed on Douglas-fir, white fir, and grand fir. Larvae secondarily attack ponderosa pine, western hemlock, and western larch after the preferred hosts have been defoliated. The population density of this moth varies tremendously over cycles of 2 or 3 years, probably due to a viral infection of the moth (USDA, 1973a).

The spruce budworm is also indigenous to North America and has caused extensive damage to conifers in Canada and the United States. In the eastern United States and Canada, balsam fir is the preferred host species, followed by white, red, and black spruce. Spruce budworm populations do not fluctuate as extremely as do those of the Douglas-fir tussock moth (USDA, 1975). In the State of Maine, the area infested with the spruce budworm increased from about 24,000 hectares (60,000 acres) in 1968, to 2.8 million hectares (7 million acres) in 1975. Batzer (1973) reported that over a 10-year period, total losses of balsam fir and spruce timber were equivalent to 12.4 cubic meters per hactare per year (1.37 cords per acre per year). Craighead (1923) estimated that a total of more than 97 million cubic meters (27 million cords) were destroyed in Maine during the outbreak of 1910 to 1918. According to the U.S. Forest Service, present conditions in northern Maine are similar to those during the budworm epidemic of 1910 to 1918.

The extensive damage to forests by defoliating insects has become a problem of national significance. The gypsy moth, spruce budworm, and Douglas-fir tussock moth have caused annual losses estimated to be greater than 68 million cubic meters (2.4 billion cubic feet) of lumber, worth about $5,000,000,000. Reforestation of these areas is expected to take more than 20 years. Thus, it is important to protect these resources from excessive insect damage, to protect important wildlife habitat, and to maintain the recreational and aesthetic values of these forest areas (USDA, 1975). However, streams in forest areas also support important fisheries, particularly trout. Some watersheds of the northeast are drained by major spawning and nursery streams for brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). Therefore, the Fish-Pesticide Research Laboratory of the U.S. Fish and Wildlife Service is cooperating with the U.S. Forest Service in investigating the potential toxicological effects of candidate forest insecticides on fish and aquatic invertebrates. The investigations were designed to evaluate changes in toxicity due to various water qualities associated with biogeographic regions. The investigations also included the toxicity of the candidate insecticides to aquatic invertebrates, to early life stages of brook trout, the possible toxic interaction of insecticide combinations, and the susceptibility of fish containing residues of DDT, or a polychlorinated biphenyl (PCB; Aroclor 1254). This paper reports progress in our cooperative research.

MATERIALS AND METHODS

Six experimental forest insecticides, Sumithion®, carbaryl, Dylox®, Matacil®, Dimilin® (TH-6040), and Orthene® were provided by various chemical companies. Stock solutions of the candidate insecticides (technical grade) in reagent grade acetone were prepared immediately before each static test. Stocks of field formulations were prepared by dilution with distilled water.

Test waters of different chemical characteristics were formulated from deionized water of at least 1 million ohms resistivity by adding reagent grade salts (Marking, 1969). Mineral acids and bases were used to buffer, adjust, and maintain pH (Marking and Dawson, 1972). Various test temperatures were controlled by water baths.

Fish obtained from Federal and State hatcheries were maintained for 2 weeks under standard fish cultural care (Brauhn and Schoettger, 1975). They were acclimated to test conditions of temperature and water quality before the experiments and subsequently transferred to test containers about 24 hours before addition of the toxicant (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). Fish used in this investigation were brook trout and Atlantic salmon. Mature scuds (*Gammarus pseudolimnaeus*) and late instar naiads of a stonefly (*Pteronarcys californica*) were used in the toxicity tests for invertebrates. The invertebrates were obtained from wild populations in streams and maintained in the laboratory (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975).

Toxicity data were analyzed with the statistical method described by Litchfield and Wilcoxon (1949) to determine the LC_{50} (concentration producing 50% mortality) and 95% confidence interval. Estimations of toxic chemical interactions were made with a modification of the methods developed by Marking and Dawson (1975). With this method, toxicities of paired insecticide mixtures were determined in the manner of the individual chemicals, except that the two chemicals were added as fractions of their activity (96-h LC_{50}'s), and in a 1 to 1 ratio of their independent toxicities. Toxicity of the mixture of chemicals was computed by summing the LC_{50} ratios of the combined to the independent toxicities of each chemical in the mixture, and calculating an interaction index (see footnote, Table 3). Evaluations of potential additive activity, and activity greater or less than additive activity were made by computing a range of index values for the 95% confidence intervals around LC_{50} values.

RESULTS

Acute Toxicity

Fish--

Toxicities of the candidate forest insecticides ranged between 0.5 to 11 mg/l (96-h LC_{50}'s), with exception of Orthene and the field formulation of Matacil (Table 1). Orthene was the least toxic; up to 100 mg/l did not kill fish within 96 h. The toxicities of Sumithion and Orthene (both organophosphate insecticides) were apparently unaffected by changes in temperature (7-17 °C), water hardness (40-320 mg/l, as $CaCO_3$), or ph (6.5-9.0). Further, the toxicities of these two field formulations were similar to those for their technical forms. In contrast, the toxicity of technical grade carbaryl, a carbamate insecticide, was influenced by water quality. It was 3 times more toxic to brook trout at 17 °C than at 7 °C; and was most toxic in hard, alkaline water, e.g., it was 4 times more toxic at pH 9.0 than at pH 6.5 (Table 1). The field formulation of carbaryl was tested for toxicity. Because it is not soluble in acetone or water, there were no mortalities, and its toxicity could not be reasonably established with these techniques.

Matacil is also a carbamate insecticide, and, like carbaryl, its toxicity was appreciably influenced by temperature and pH, but not by water hardness (Table 1). It was over 3 times more toxic at 17 °C than at 12 °C, and over 6 times more toxic at pH 9.0 than at pH 7.5. However, the most significant finding from these tests was the discovery that the field formulation of Matacil (17% active ingredient) was as much as 70 times more toxic than its technical form and could pose a serious hazard to trout.

The toxicity of the organophosphate insecticide Dylox to brook trout was influenced by temperature, water hardness, and pH (Table 1). The toxicity was 18 times greater at 17 °C than at 7 °C; almost 10 times greater at pH 8.5 than at pH 6.5; and about 4 times more toxic in very hard water (320 mg/l) than in soft water (40 mg/l). The toxicity of the field formulation of Dylox (40% active ingredient) was similar to that of the technical grade material.

Dimilin is a comparatively new growth regulator that inhibits chitin synthesis during the molting of immature insects. No extensive toxicological tests have been conducted with this compound, but preliminary data indicate that it is relatively non-toxic to fish.

TABLE 1. ACUTE TOXICITY[1] OF SIX CANDIDATE FOREST INSECTICIDES TO BROOK TROUT IN WATER OF DIFFERENT TEMPERATURE, HARDNESS, AND pH.

Compound (% active ingredient	Test Water Temp. (C)	Water hardness (mg/l as $CaCO_3$)	pH	96h-LC_{50} and 95% confidence interval (mg/l)
Carbaryl (Technical, 99.5%)	7	40	7.5	3.0 2.0-4.5
	12	40	7.5	2.1 1.7-2.6
	17	40	7.5	0.9 0.7-1.3
	12	40	8.0	5.4 4.5-6.5
	12	320	8.0	2.5 1.8-3.5
	12	40	6.5	4.6 3.5-6.0
	12	40	7.5	3.7 2.0-6.7
	12	40	8.5	2.1 1.7-2.6
	12	40	9.0	1.1 0.8-1.6
(Field formulation, 49%, Sevin-4-Oil®)	12	40	7.5	—[2] — —

(continued)

TABLE 1 (Continued)

Compound (% active ingredient	Test Water Temp. (C)	Test Water Water hardness (mg/l as $CaCO_3$)	Test Water pH	96h-LC_{50} and 95% confidence interval (mg/l)
Metacil (Technical, 98%)	7	40	7.5	9.4 6.9-13
	12	40	7.5	9.0 6.2-13
	17	40	7.5	2.6 2.1-3.2
	12	40	8.0	8.0 5.8-11
	12	320	8.0	11 8.7-13
	12	40	6.5	8.6 5.0-15
	12	40	7.5	9.0 6.2-13
	12	40	8.5	7.8 5.2-12
	12	40	9.0	1.4 0.9-2.1
(Field formulation, 17%)	12	40	7.5	0.13 0.10-0.17

(Continued)

TABLE 1 (Continued)

Compound (% active ingredient	Test Water Temp. (C)	Test Water Water hardness (mg/l as $CaCO_3$)	Test Water pH	96h-LC_{50} and 95% confidence interval (mg/l)
Dylox (Technical, 99%)	7	40	7.5	9.4 7.9-11
	12	40	7.5	2.5 2.2-2.9
	17	40	7.5	0.50 0.38-0.65
	12	40	8.0	2.4 2.1-2.8
	12	320	8.0	0.62 0.49-0.79
	12	40	6.5	9.2 7.8-11
	12	40	7.5	4.4 2.9-6.5
	12	40	8.5	0.96 0.78-1.2
(Field formulation, 40.5%)	12	40	7.5	5.5 4.6-6.58
Orthene (Technical, 94%)	7	40	7.5	>100
	12	40	7.5	>100
	12	40	7.5	>100

(Continued)

TABLE 1 (Continued)

Compound (% active ingredient	Test Water Temp. (C)	 Water hardness (mg/l as $CaCO_3$)	 pH	96h-LC_{50} and 95% confidence interval (mg/l)
Orthene (Technical, 94%)	12	40	7.5	>100
	12	320	7.5	>100
	12	40	6.5	>100
	12	40	9.0	>100
(Field formulation, 75%)	12	40	7.5	>100
Sumithion (Technical, 95%)	12	40	7.5	1.7 1.4-2.1
(Field formulation, 40%)	7	40	7.5	2.1 2.0-2.5
	12	40	7.5	2.2 2.0-2.5
	17	40	7.5	1.8 1.5-2.0
	12	40	8.0	2.0 1.7-2.4
	12	320	8.0	1.4 1.1-1.7

(Continued)

TABLE 1 (Continued)

Compound (% active ingredient	Test Water			96h-LC_{50} and 95% confidence interval
	Temp. (C)	Water hardness (mg/1 as $CaCO_3$)	pH	(mg/1)
Sumithion (Field formulation, 40%)	12	40	6.5	1.7 1.4-1.9
	12	40	7.5	1.7 1.3-2.1
	12	40	8.5	1.5 1.1-2.1
	12	40	9.0	1.6 1.2-2.0
Dimilin (Field formulation, 25%)	10	40	7.5	>100

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[1] Toxicity based on active ingredient in static tests.

[2] Not determined.

TABLE 2. ACUTE TOXICITY[1] (96-h LC_{50} AND 95% CONFIDENCE INTERVALS, mg/l) OF SIX CANDIDATE FOREST INSECTICIDES TO MATURE SCUDS AND STONEFLY NAIADS TESTED AT 18 °C and 16 °C

Compound (% active ingredient)	Invertebrate and Test Temperature	
	Scud 18 °C	Stonefly 16 °C
Sumithion (95%)	>0.01	0.004 0.002-0.006
Matacil (98%)	0.012 0.008-0.018	
Dylox (99%)	0.040 0.026-0.060	0.035 0.022-0.055
Carbaryl (99.5%)	0.016 0.012-0.019	0.002 0.001-0.003
Orthene (94%)	>100	
Dimilin (25%)	0.030 0.020-0.050	

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[1] Toxicity based on active ingredient in static tests.

[2] All chemicals tested were technical grade material except the field formulation of Dimilin.

Invertebrates--

The 96-h LC_{50}'s for scuds and stoneflies were in the range of 0.002 to 0.04 mg/l of the insecticides tested, with the exception of Orthene (Table 2). In general, invertebrates appear to be about 100 times more susceptible to these chemicals than are brook trout. Although Dimilin was not toxic to brook trout, it was highly toxic to scud. Of all six compounds, Orthene was by far the least toxic to both brook trout and the aquatic invertebrate tested.

Toxicity to Eggs and Sac-Fry

To date, studies of the effects of insecticides on life stages of brook trout have been completed only for Sumithion. Eyed eggs (2 days before hatch) were exposed to concentrations of Sumithion similar to those detected in Maine streams after experimental aerial applications of the compound (Marancik, 1976). These tests were conducted in a flow-through system described by Mount and Brungs (1967), and test concentrations were reduced by one-half for 4 consecutive days and then stopped. For example, with the highest concentration tested, eggs were exposed to 0.1 mg/l on day 1, 0.05 mg/l on day 2, and the sac fry to 0.025 mg/l on day 3, and 0.012 mg/l on day 4. Treatment was then stopped, but the fry were observed for an additional 30 days to monitor anomalies or delayed mortality. The Sumithion concentration of 0.1 mg/l, which was several times greater than the highest post-treatment concentration reported by Maranick in streams, did not significantly ($p=0.05$) affect survival or development of brook trout sac fry.

Chemical Interactions

The U.S. Forest Service anticipates that several of the candidate forest insecticides may be applied to forests at about the same time, concurrently for different pests, or that applications of different compounds to infested areas may overlap. In addition, fish such as Atlantic salmon and brook trout containing background residues of DDT and PCB's may also be exposed to Guthion (an organophosphate insecticide), which is used in blueberry culture. Therefore, the likelihood of salmon and trout receiving multiple chemical exposures is high, and potential toxic interactions must be explored.

Insecticide Interactions--

Brook trout and Atlantic salmon were exposed to paired mixtures of candidate forest insecticides, or to a combination of Dylox and Guthion. The toxicities of all insecticide combinations were simply additive, except for the Dylox and Guthion mixture which was synergistic in brook trout and Atlantic salmon (Tables 3 and 4). This synergistic action of Dylox and Guthion was also observed by Marking and Mauck (1975) in studies with rainbow trout (*Salmo gairdneri*).

Insecticide, DDT, and PCB Interactions--

The carbamate insecticides carbaryl and Matacil were about twice as toxic to brook trout containing Aroclor 1254 (PCB) residues of 2.3 µg/g as compared with trout containing lower residues (Table 5). However, there appeared to be no interaction of the organophosphate insecticides Dylox and Sumithion with Aroclor 1254 residues in this species.

Mixtures of Dylox and Guthion were essentially as synergistic to Atlantic salmon as they were to brook trout, but young salmon containing total body residues of 1.5 μg/g DDT (and its analogs) were no more susceptible to the mixture than were those without residues (Table 4). Aroclor 1254 residues of 2.3 μg/g in brook trout did not appreciably alter the relative synergism of the Dylox with Guthion, or the additive toxicity of any of the other forest insecticide mixtures (Table 3).

DISCUSSION

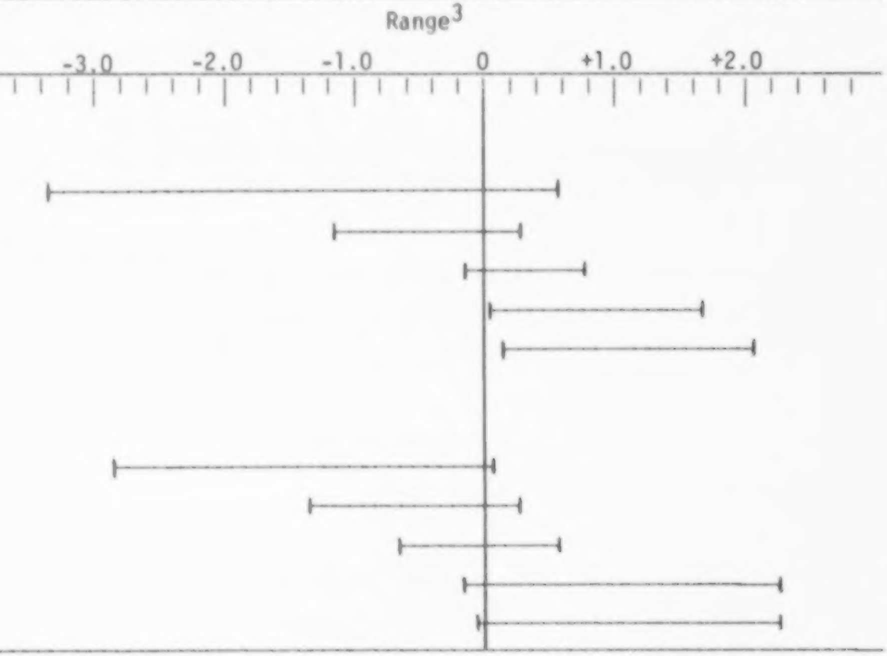
Sumithion, carbaryl, Dylox and Matacil are all relatively much more toxic to young brook trout and Atlantic salmon than are Orthene and Dimilin. However, barring accidental spills or excessive or overlapping applications, the toxic concentrations determined (with one exception) are well above those measured in streams after experimental aerial applications. The exception, the liquid field formulation of Matacil, was 10 to 70 times more toxic to brook trout than its technical grade form, and concentrations exceeding the 96-h LC_{50} of 0.13 mg/l could be expected in streams after aerial applications. The hazard connected with use of this formulation would be increased in alkaline waters. Alkaline pH and high water temperatures increased the toxicity of several of the candidate forest insecticides to fish, but for most of these chemicals, even this elevated toxicity does not appear sufficient to pose a significant toxicity hazard.

Although some of the candidate forest insecticides appear to be synergized by other insecticides, this reaction is not likely to be an imminent hazard to brook trout and Atlantic salmon, unless streams receive far greater doses of the chemicals than have thus far been measured in experimental aerial applications. A similar relationship exists in fish containing Aroclor 1254 residues, but the relatively toxic field formulation of Matacil could be even more toxic to fish containing significant Aroclor residues. Chemical interactions in invertebrates were not tested. However, considering the high toxicity of all of the compounds except Orthene to these organisms, synergistic interactions could have a dramatic effect.

The high susceptibility of scuds and stonefly naiads to Sumithion, carbaryl, Dylox, Matacil, and Dimilin suggests that aquatic invertebrates are much more sensitive to these compounds than are fish. In addition, the LC_{50} values appear to be well within the concentrations measured in streams after experimental aerial applications. Insecticide concentrations of 0.24 mg/l (Haugen, 1976) to 0.013 mg/l (Marancik, 1976) have been measured at 20 minutes and 24 hours, respectively, after application. However, Orthene should not pose a significant toxicity hazard to fish or invertebrates.

TABLE 3. EFFECT OF PCB (AROCLOR 1254) RESIDUES ON SENSITIVITY[1] OF BROOK TROUT TO CANDIDATE FOREST INSECTICIDES

PCB residues in trout and insecticide mixture tested	Interaction index[2]
Aroclor 1254 residues, 0.08 μg/g[4], or less	
Carbaryl, Dylox	-0.68
Carbaryl, Sumithion	-0.30
Sumithion, Dylox	+0.33
Guthion, Dylox	+0.65
Guthion, Dylox	+0.93
Aroclor 1254 residues, 2.3 μg/g[4]	
Carbaryl, Dylox	-0.88
Carbaryl, Sumithion	-0.29
Sumithion, Dylox	+0.10
Guthion, Dylox	+0.67
Guthion, Dylox	+0.70



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[1] Tests conducted in standard reconstituted water at 12 °C.

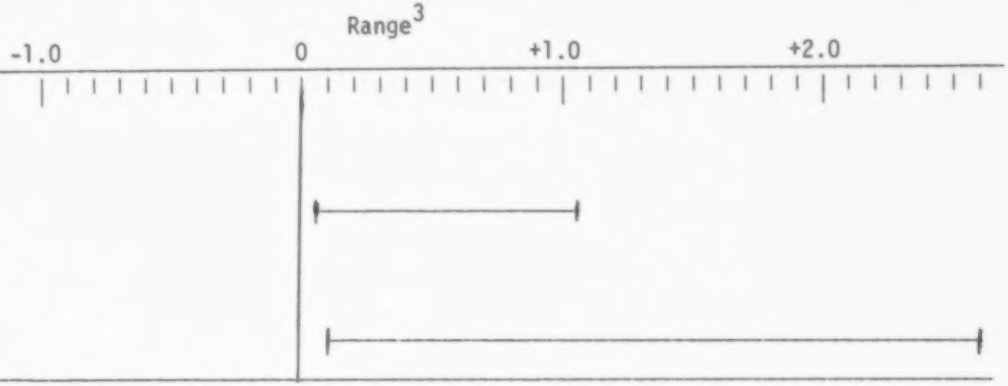
[2] Interaction Index (I.I.) = $\frac{Am\ (LC_{50})}{Ai\ (LC_{50})} + \frac{Bm\ (LC_{50})}{Bi\ (LC_{50})}$ where Ai and Bi are independent LC_{50}'s of insecticides A and B, and Am and Bm are LC_{50}'s for A and B calculated from tests of the mixture.

[3] Ranges that cross the zero line indicate addivitive toxicity; ranges on the positive side indicate greater than additive toxicity; and ranges on the negative side indicate less than additive toxicity.

[4] Mean total body residues.

TABLE 4. EFFECT OF DDT RESIDUES ON SENSITIVITY[1] OF ATLANTIC SALMON TO A MIXTURE OF GUTHION AND DYLOX

DDT residues in salmon	Interaction index[2]
DDT residues, less than 0.01 μg/g	
mixture	+0.56
DDT residues, 1.5 μg/g	
mixture	0.95



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[1] Tests conducted in standard reconstituted water at 12 °C.

[2] Interaction index (I.I.) = $\frac{\text{Am } (LC_{50})}{\text{Ai } (LC_{50})} + \frac{\text{Bm } (LC_{50})}{\text{Bi } (LC_{50})}$ where Ai and Bi are independent LC_{50}'s of insecticides A and B, and Am and Bm are LC_{50}'s for A and B calculated from tests of the mixture.

[3] Ranges that cross the zero line indicate additive toxicity; ranges on the positive side indicate greater than additive toxicity; and ranges on the negative side indicate less than additive toxicity.

[4] Mean total body residue of DDT, including DDT, DDE, and DDD.

TABLE 5. EFFECT OF PCB (AROCLOR 1254) RESIDUES (TOTAL BODY) ON SENSITIVITY OF BROOK TROUT TO FOUR FOREST INSECTICIDES

Insecticide	Residues of Aroclor 1254 in fish (μg/l)	96-h LC_{50}[1] and 95% Confidence Interval (mg/l)
Carbaryl	0.08[2]	5.9 5.1-6.9
	0.4	5.0 4.1-6.0
	2.3	2.5 1.7-3.7
Matacil	0.08[2]	11 8.8-13
	0.4	10 7.7-14
	2.3	6.5 4.5-9.0
Dylox	0.8[2]	3.8 3.1-4.6
	0.4	4.6 3.6-5.8
	2.3	3.9 3.0-5.1
Sumithion	0.08[2]	1.6 1.4-1.7
	0.4	1.5 1.3-1.7
	2.3	1.7 1.5-2.0

[1] Toxicity based on active ingredient; tests conducted with 0.3-g fish at 12 °C.

[2] Background Aroclor 1254 residues.

In summary, aerial application of the six potential DDT substitute forest insecticides, with the exception of the field formulation of Matacil, should not have a major toxic effect on brook trout and Atlantic salmon. Marancik (1976) reported some inhibition of brain cholinesterase in fish after aerial applications, but enzyme activity returned to normal within 48 h. Nevertheless, all of the candidate insecticides, except Orthene, may kill aquatic invertebrates. Results published by others (e.g., Burdick *et al.*, 1960; Elson *et al.*, 1973; Flannagan, 1973) showed that populations of stream invertebrates were markedly reduced after aerial applications with forest insecticides other than DDT. Therefore, field investigations are needed to determine whether any of the present insecticides significantly depress aquatic invertebrate populations, and, if so, the duration of the population depressions. The timing of such effects on invertebrates may be critical with respect to adequate food supplies for young salmon and trout.

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SECTION 4

PRINCIPLES AND METHODS OF BIOLOGICAL ESTABLISHMENT OF THE NORMS OF CHEMICAL SUBSTANCES AND EVALUATION OF THE LEVEL OF POLLUTION IN WATER-BODIES

V.I. Lukyanenko

Among the most important of the present problems is the question of "clean water", i.e., the protection of waters from chemical pollution in order to preserve the biological processes associated with a high quality of water. This problem is extremely acute, complex, and enormous by its scale. In this regard, it is sufficient to recall that from 450 to 700 km^3 of waste water is discharged annually, the greater fraction of which undergoes either no or only partial treatment. Reliance is placed jointly upon the dilution of the waste waters with clean river waters and the process of self-purification. However, to neutralize even 450 km^3 of waste water, provided at least a half of it undergoes treatment, a total of 6,000 km^3, or almost 40% of the so-called stable river discharge of the globe will be required. For this reason, the prediction of the Institute of Geography of the Academy of Sciences of the USSR seem to be quite real. This Institute suggests that by the year 2000, all the water of rivers will have to be used for neutralization of waste waters, even if the sewage be treated by more perfect methods.

It is necessary to account for the fact that the pollution of waters accelerates, but their self-purification capacity declines. Therefore, one can not plan to increase the "toxicologic load" on rivers. On the contrary, the general way to solve the problem of "clean water" is to reduce this load by building sewage treatment plants and raising their efficiency. This will enable a decrease in the amount of clean water required for dilution of wastes, and provide for optimum functioning of the ecosystems responsible for "self-purification" of waters. Thus, exhaustion of water resources in the nearest future can be avoided.

The urgency of this task of improving treatment of industrial wastes and increasing the efficiency of sewage treatment plants is determined not only by the high toxicity of many hundreds of chemical substances contained in waste waters. The fact is that the volume of discharge from arable lands containing various pesticides has greatly increased for the last two decades. This discharge enters the same waters which receive domestic and industrial wastes. Consequently, the "toxic" load on natural waters becomes greatly increased. The principal way of preventing pollution of waters by toxic industrial wastes is the treatment of the sewage and limitation of its discharge into receiving waters. This way is not applicable to the diffuse discharge from the agricultural lands. Here the method is to determine the toxicity for aquatic organisms of each of the respective toxicants, and prohibit or restrict the use of pesticides characterized by a very high toxicity to fishes and aquatic invertebrates. The list of pesticides used in modern agriculture is extensive, and the search for new ones is so rapid that thousands of herbicides alone are patented every year. It is not difficult to realize how labourous the task of preliminary determination of toxicity of new pesticides is, especially if one accounts for the present empirical approach of the water toxicologists to its solution.

Currently, aquatic toxicology does not enjoy the general theory of the action of pesticides on a living cell and the organism as a whole. In both domestic and foreign literature, there are still very few studies devoted to the mechanisms of action of toxicants upon the cell, subcell, and molecullar structures. Without an understanding of these mechanisms, it is impossible to comprehend the development of toxicologic processes brought about by different groups of toxicants. Nevertheless, it is this understanding of toxicologic processes that must be the basis for choosing the methods of evaluation of the toxicity of a substance, or a group of structurally related substances. This understanding is also essential for the determination of the maximum permissable concentrations (MPC) of these substances in natural waters. The accepted practice in the USSR demonstrates that one of the most efficient means of providing protection of waters from pollution is hygienic and fisheries standardization, i.e., the establishment of maximum permissible concentrations (MPC) for toxic substances entering water bodies. It is not merely coincidence that both the medical profession and biologists have arrived at this solution. Ichthyologists, hydrobiologists, sanitary and hygiene medical personnel face the same problem, i.e., assuring clean natural waters, preventing such a degree of pollution as to cause poisoning of animals and human beings, and alteration of the normal course of biological processes determining productivity of the waters, and their "self-purification" which render water drinkable. It is quite understandable that the degree of toxicity of a substance for animals, fish, and aquatic invertebrates can be established only under experimental conditions. Harmless concentration of a given substance for a given organism may be found in the same way. The sensitivity and resistance of various land animals to toxic substances is not the same as for aquatic organisms, thus, the sanitary-hygienic and fisheries requirements for quality of water related to toxic substances are different.

Experimental data accumulated to date clearly show that the values of the MPC of many substances for aquatic organisms, especially for fishes (fisheries MPC), are lower, i.e., more "stringent" than for warmblooded animals and humans (Sanitary-hygienic MPC). For example, the fisheries MPC of copper (0.01 mg/l), nickel (0.01 mg/l) and zinc (0.01 mg/l) are only one hundredth as high as the sanitary-hygienic MPC of the same metals (1 mg/l). Similary, the toxicity of many organic substances, for fishes and aquatic invertebrates (especially pesticides) is hundreds of times as high as that of the warmblooded animals. The cause of these differences is quite evident. The warmblooded animals undergo a short-term contact with the polluted water which enters them in relatively small portions, while the water is the permanent home of the aquatic organism.

Does it mean that the criteria for toxicity and methods of estimation of toxic effect of various chemical substances elaborated by general and sanitary toxicology are not applicable to one of the classes of vertebrates - fishes, and to aquatic invertebrates? Of course it does not. The author (1973) has previously emphasized that toxicology of fish is a part of comparative and general toxicology. In connection with this, many ideas and methods may be used in solving the practical tasks of aquatic toxicology associated with protection of waters from chemical pollution. The last ten years of impetuous development of ichthyo-toxicological investigations both in our country and abroad have yielded confirmation of fruitfulness of this point of view. Today we can take the next step on the way to consolidation of the efforts of medical man and biologists in solution of the problem of "clean water".

The unity of aims of both the sanitary-hygienic and fisheries personnel in establishment of standards for chemical substances discharged into natural water (i.e., preservation of clean water in rivers and reservoirs) conditions the necessity for creative analysis of the principles of establishment of standards and elaboration of the universal system of the MPC. This system must enable protection of water bodies from ecological sanitary-hygienic and fisheries point of view. In essence, from a biological approach. After all, the cleanliness of water depends upon the biological processes of production and distruction; the dynamic equilibrium which determines the high quality of "living" water.

Within the foundation of the biological establishment of standards for the MPC of chemical substances must lie the main elements of sanitary-hygienic and fisheries principals established at the present time. This includes the evaluation of the effect of chemical substances on organoleptic properties (taste and smell) of water and aquatic organisms, the sanitary condition of the water-body (processes of mineralization of organic pollution), the toxic action of the incoming substances upon aquatic organisms of different levels of organization, and the effect upon laboratory animals which are used by medical personnel for determining sanitary-toxicological harmfulness.

When expansion of the unit biological standard of the MPC is considered, the generally accepted methodical scheme of the sanitary-hygienic setting establishment of standards of the MPC will not suffer any essential change, since its validity is established. It is only necessary to expand the genetic aspects of investigations, since many chemical substances entering the waters with sewage are genetically active, i.e., capable of bringing about both mutations and modifications in concentrations that are significantly lower than those established for the hygienic MPC (Rapoport, 1972). Genetic activity of toxicants (induction of genetic mutations; aberration of chromosomes) is manifested at such a level that is is impossible to evaluate these changes with the common physiological and biochemical tests. The main object of investigations of the mutagenic and morphogenic activity of toxicants must become, in the view of I.A. Rapoport (1972), more intensively studied by genetics, e.g., *drosophila sp.* being a likely choice since it possesses, like humans, the nucleoprotein genom, but the number of genes is only one tenth - one twentieth as great. There is good cause to agree with I.A. Rapoport (1972) when he states, "genetic experiments on *drosophila* provide a unique possibility to determine the ability of the chemical agents to induce mutation in genes, injure chromosomes, as well as assess the influence of pollutants upon moving apart the chromosomes, the latter being a very important parameter of the genetic danger of chemical pollutants in the environment".

The scheme of the fisheries MPC must be reviewed and modernized in two directions. First, it is necessary to pay more attention to the biological aspects of setting of the standards of harmful substances entering water-bodies, and to evaluation of the efficiency of this process. It must be emphasized that here two different "ecological" aspects are addressed: (1) the ecological foundations of the biological establishment of the standards of the MPC, and (2) the ecological foundations of evaluation of the efficiency of this process, directly on water-bodies.

The first of the two aspects has been considered by the author (1967) in detail at an earlier date. On the basis of that experimental data, and data from the literature, a number of propositions about the importance of the role of ecological (abiotic) factors of aquatic environment for determining sensitivity and resistance of aquatic organisms to toxic agents have been formulated. The propositions include the necessity of considering this dependence when the MPC is established. Physical and chemical properties of the water medium influence the latent period, dynamics of intoxication, and the threshold of resistance of fishes and other organisms to poisons. In other words, the actual toxicity of some poisons (ions of heavy metals, acids, alkalis, and organic poisons) may be either reduced or intensified depending upon the environmental background.

There are two principal routes of influence of physical and chemical parameters of the water medium upon the toxic-resistance of aquatic organisms: (1) direct, and (2) indirect. The first is a direct influence upon the living organism, by changing the level of metabolism which leads to an increase in the toxic-resistance. Changes in the normal regime of functioning of different physiological systems, particularly the onset of extreme conditions (high temperatures, rapid fluctuations in temperature, oxygen deficiency, etc.) lead: (1) to easier penetration and accumulation of toxicants in an organism; (2) to destruction or weakening of the detoxification mechanisms and the processes of releasing of the toxicants from the organism; (3) to increase in sensitivity of some functional systems (target functions) to toxic substances; and (4) to a decrease in resistance. Any combination of these changes, or an individual change alone, can reduce the total resistance of the organism, and thus lead to a greater toxic effect for a given chemical agent or combination of substances.

The second route of influence of the abiotic factors of environment upon the resistance of the aquatic organism to poisons is the indirect mechanism, a factor which often decreases the actual toxicity of a substance. By this is meant a change in the toxicity of the substance owing to decrease of its actual concentration in solution, or its physical and chemical transformation. The decrease in the toxicity of many heavy metal ions in hard water and in sea water due to formation of precipitates is illustrative of this mechanism. A change in the toxicity of various metals as a result of a complete or partial hydrolysis, formation of poorly soluble carbonates, and precipitation from solutions having the pH value far from neutral is also well known.

Experimental data on the dependence of the degree of toxicity of various substances and resistance of aquatic organisms (mostly fish) upon ecological factors are long known, although these data are still not used in establishing the MPC. However, a skillful use of existing information when determining the MPC of a given substance would allow not only a reduction of the duration of experiments by conducting them under extreme conditions (high temperatures, low oxygen content, etc.), but would also invest the proposed MPC with an "ecological factor of safety", i.e., with due consideration of the range of fluctuations of physical and chemical factors of the environment.

Such an "ecological MPC" would guarantee the relative well-being of the ecosystem as a whole, as well as its separate components. It would protect the components from poisoning even under conditions of deterioration of the main abiotic factors which usually leads to a decrease in the toxic-resistance of aquatic organisms.

Another aspect of the so-called ecological setting of standards for harmful substances in water became an object of special discussions only recently. By this is meant the so-called ecological MPC designed to secure cleanness or "health" of a water body as a whole, i.e., preservation of natural ecosystem of the water-body and not only important commercial organisms. However, the attempts made to clarify this concept lead to the conclusion that one must speak not of "ecological MPC", but of ecological foundations of establishing the MPC as noted by M.M. Kamshilov, "Determination of concentrations of foreign substances not disturbing natural biological circulation in aquatic ecosystem". The fisheries MPC are, in essence the "ecological MPC", since they must secure protection from toxicants of not only fish, but also the ecosystem as a whole.

It is quite a different matter, when we speak of search and standardization of the indices of "ecological well being" which are very important for the estimation of the efficiency of biological standard setting relative to toxicants discharged into waters. The investigation of the polluted water bodies of the effect of domestic and industrial wastes on the ecosystems of these water-bodies has been performed by sanitary hydrobiology. Aquatic toxicology, including ichthyo-toxicology was born as an outgrowth of sanitary hydrobiology.

The development of these disciplines became possible as a result of the realization of the fact that no investigation and no description of the changes occurring in the communities residing in polluted waters, regardless of how thorough they might be, was able to address the issues of which components were involved, and what degree of removal would be required. These questions could only be answered with a help of experimental methods of investigation and establishment of the MPC.

The impetuous development of aquatic toxicology and ichthyo-toxicology during the last two decades has lead to a notable decline in sanitary-hydrobiological investigations. However, the main object of these investigations remains the water-bodies polluted with organic matter.

Herein lies one of the reasons behind the development of the interest of some toxicologists in ecological aspects of aquatic toxicology which must actually be dealt with by sanitary hydrobiology. A distinct demarkation of the tasks and methods of sanitary hydrobiology and aquatic toxicology is needed not only for successful solution of specific problems faced by each of the sciences, but also for establishment of fruitful contacts when solving the problems of protection of waters from pollution. It is to be emphasized that the evaluation of the effectiveness of biological setting of standards for substances discharged into waters can be exercised only from the criteria and methodology of sanitary hydrobiology, which has as its object of study the water-body as a whole and its living communities.

In this regard, the indices of ecological health imposed by M.M. Kamshilov, e.g., the oxygen concentration in the water; ratio of production to destruction; character of benthic communities; and the distribution of indicator species; deserve deep attention. The organoleptic symptom of harmfulness must also be included. This symptom implies the influence of pollution on organoleptic properties of not only the water, but also of the aquatic organisms, including fish. Unfortunately, this aspect of fisheries investigation attracts scant attention in comparison with the sanitary-hygienic setting of standards. It is enough to recall that out of 420 established sanitary-hygienic MPC standards of harmful substances, more than a half (216) are limited by the organoleptic index, 147 by sanitary-toxicologic considerations, and 57 substances by the general sanitary index of harmfulness. But within the fisheries MPC, only 15 substances of a total of 70 are limited by the organoleptic index. The main reason for the poor use of the organoleptic index in the fisheries MPC is an insufficient elaboration of the methods of objectively evaluating the reaction of aquatic organisms, including fish, to changes in taste and smell of the water caused by toxic substances. The methods of investigation of these reactions exist, in the form of conditioned reflexes which still awaits wide application in ichthyological investigations.

Nevertheless, the sensitivity of fish to the odors of many chemical substances greatly exceeds that of a human being. Thus, Hasler and Wisby (1951) discovered in fish the ability to detect phenol in concentrations 0.01 mg/l, or even 0.005 mg/l, using a conditioned reflex. These concentrations are considerably lower than the threshold for humans. These data agree with the results of Neurath (1949) who reported that fish detect the the smell of phenethyl alcohol at the concentrations 250 times lower than humans. An even greater sensitivity of the eel to beta-phenethyl alcohol was demonstrated by Teichmann (1957). The eel showed a reaction to this substance at concentrations as low as 3×10^{-20} mg/l, i.e., when only 2-3 molecules of the substance could be present in the olfactory bulb. The fish discern perfectly well the smells of many aquatic plants (Walker and Hasler, 1949), as well as the smells of fish and other vertebrates (Von Frisch, 1941; Sliultz, 1956; Walker and Hasler, 1949). In most cases the sensitivity of fish to the smells of substances excreted by closely related species is greater than to those of taxonomically remote species. The repellent effect on fish may be produced by substances excreted from the skin of other classes of vertebrates. Thus, buffotoxin extracted from the skin of adult toads is preceived by fish, and produces a repellent effect even at a dilution of $1:2.4 \times 10^6$. These are but a few of the reported studies noting the extremely high sensitivity of fish to changes in smell and taste of water which they inhabit.

Such a high sensitivity of fish to the organoleptic properties of water can not but affect the distribution of fish in a water-body. It is not difficult to imagine that tens and hundreds of substances, mostly of an organic nature, entering natural waters with sewage may produce a repellent or an attracting effect on fish changing feeding, wintering, and spawning conditions; causing unnaturally high concentrations of fish in a limited area, driving fish away from food, and thus making it difficult to use the nutrient base, and reducing the bio-productivity of a whole water-body. All of these complicated and intricate manifestations of the "ecological ill-health" of a water body receiving organic substances, even in strict conformity with the established standards, may be properly considered only on the basis of the knowledge of the reactions of avoidance of the poisons, which change taste and smell of the water and food organisms. Therefore, one of the main tasks in the field of experimental aquatic toxicology is the thorough study of behavioral reactions. This study must include the reactions of detection and avoidance of chemical agents, the study of mechanisms and the character of these reactions (repellent or attracting), and the resolving power of the olfactory and gustatory organs in fish. In summary, the idea is to establish physiological foundations for a wide application in biological standards which have been well established in the sanitary-hygienic standards related to chemical pollution of water-bodies. At the same time, the study of avoidance reactions in fish will enable an understanding of the peculiarities of distribution of fish in water-bodies which are "loaded" with waste waters in accordance with the biological standards. It should be noted that the distribution of indicator organisms, fish included, may serve as one of the indices of "ecological health" of a water-body. Thus, reference is made to the "ecological establishment of the standards" or, to be more correct, to the ecological principles of evaluation of the efficiency of biological setting of standards.

So, the ecological principle is very important for the evaluation of the effectiveness of biological setting of standards for substances entering waters. Even the most thorough observations and detailed descriptions of the changes in aquatic ecosystems are not able to reveal harmless concentrations of chemical substances discharged into a water-body, or to establish what substance or a group of substances cause the noticed changes in the ecosystems. Similarly, the study of the most important physiological and biochemical parameters of various aquatic organisms dwelling in a polluted water-body and the detection of essential disturbances in functioning of living systems do not reveal harmless concentrations of chemical agents. Therefore, the main task of the biological setting of standards, i.e., establishment of the MPC, may be solved only under experimental conditions on the most sensitive test-objects or representative organisms of any natural ecosystem. Of course, such a scheme of experiments, i.e., the separate use of the most sensitive elements of the ecosystems, leads to certain simplifications of the real situation in the water-body. It would be, however, a naive assumption to expect that these simplifications might be avoided by experimenting in ponds or canals rather than in aquaria, or complex "natural ecosystem" as opposed to individually sensitive test objects. This delusion comes from confusing the main tasks of the principles and methods of sanitary hydrobiology and aquatic toxicology. One should not be embarrassed by the experimentally unavoidable "simplifications" of a real situation, just as medical science is not embarrassed when sanitary-hygienic standards for chemical substance are established. The sanitary-hygienic MPCs are meant to secure the safety of human beings, but they are established in experiments using small rodents or other larger mammals (rabbit, dog).

Similarly, the genetic investigations designed to determine the MPC will most likely be performed on *drosophila sp.*, a classical test animal in genetic investigations, having a number of advantages over laboratory mammals. In this regard, the position of aquatic toxicologists is easier since the possibility of studying the MPC directly exists. This direct application also allows the selection of the most sensitive species. The use of the most sensitive and least resistance components of natural ecosystems, namely fish and aquatic invertebrates, makes the experimenting on more complex ecosystems for the establishment of the MPC unnecessary.

The concept of "natural ecosystems" itself has no single meaning, and will be essentially different for each experimental water body, to say nothing of those natural waters which serve as "receivers" of waste waters. Figuratively speaking, "the natural ecosystem" of the experimental small pond differs from "the natural ecosystem" of a reservoir or a lake to much greater extent than do small laboratory rodents from man. Consequently the appeals to change the experiments on test-objects in aquaria for experiments on "natural ecosystems" in small ponds are lacking serious scientific foundations, and do not take into consideration the needs of today's life, i.e., to establish in the shortest time the MTCs of hundreds of substances entering waters in connection with the appearance of new branches of industry, modernization of technological processes and the advances of chemistry in agriculture.

It is well known that the metazoans, which constitute the basis of grazer circulation, are more sensitive to toxicants than are the unicellular organisms. Among the metazoans, the vertebrates are more sensitive to various toxicants, specifically, organic compounds, than are the invertebrate forms. This fact served as a basis for wide use of various species of fish when establishing the MPC values with the help of the method of the so-called fish-test both in the USSR and abroad, including the USA. It should be noted that the time factor, i.e., determination of the toxicity of a substance in a shortest possible time, becomes decisive today. Therefore, the problem of rapid establishment of biological MPC of chemical substances is the number one problem in both the scientific and the commercial considerations, since the number of toxic substances discharged into the waters grows at a frightening rate.

The approaches of Soviet and American water toxicologists to solution of this problem differ primarily in the importance attached to long-term (chronic) and short-term (acute) experiments. In the USA there is a method of estimation of the conventional harmless concentration of a toxicant assumed to be 0.1 x TL (tolerance limit). The TL value is found in short-term experiments (exposure of 24-96 hours). Such an approach enables quick answers to the question of the degree of toxicity of a substance and its conventional harmless concentration. An essential disadvantage of this method is that the toxicity of many substances is displayed in prolonged (chronic) experiments at the concentrations much lower than 0.1 x TL.

The method of estimation of the MPC in the Soviet Union allows acquisition of more confident data about the toxicity of substances owing to prolonged observations of the survival of various aquatic organisms (from fish to microbes), but is is very laborous and time consuming. This makes it necessary to find a reliable, but time saving method of determination of MPC. The main direction of the search is the experimental elaboration of the transition from acutely toxic and threshold concentrations, to maximum permissible ones. Here cooperative Soviet-American investigations are needed with the application of the methods of physiological, biochemical and biophysical analysis for the quickest possible detection of the symptoms of intoxication of varying aquatic organisms.

The physiological and biochemical foundations of the determination of the MPC (Lukyanenko, 1965, 1967, and 1973) allowed the development of the method of physiological/biochemical indicators, the resolving capacity of which is tens of times higher than that of the method of the "fish-test". The method of the indicators allows detection of toxic effects of a substance by an understanding of the condition of one or another functional system of the organism. This can be done in a much shorter time with the indicator experiments than with the "fish-test", since the disturbances in functions are observed long before the lethal effect. The choice of the method of determination of the MPC of the investigated substance must be based on the knowledge of toxicological dynamics of this substance and the mechanism of its action, i.e., a clear idea of the most susceptible function or "target function" (Lukyanenko, 1973).

Determination of the MPC of chemical substances entering water-bodies is an important function, but not the only task of aquatic toxicology. The diversity and complexity of the problems faced by aquatic toxicology and ichthyo-toxicology implies a wide application of many modern methods, primarily physiological and biochemical evaluation of the toxicity of investigated substances.

Thus, it is necessary to establish and accept a unified scheme for conditioning ichthyo-toxicological investigations in order to obtain comparable data on the toxicity of the pollutants of water. It is no secret that the Mont Blanc of experimental data on toxicity accumulated in the world literature is of little value. The reason for this is simple: data from different authors are hardly comparable because of the absence of standardization in performance of the experiment and because of the lack of uniform expression of the results. Today when the agenda is international cooperation in the field of aquatic toxicology and ichthyo-toxicology, the absence of unified standard scheme for conducting toxicity experiments and determining MPC values is especially distressing.

Therefore, it is necessary to consider again the standard scheme of ichthyo-toxicological investigations (Lukyanenko, 1967, 1968) which includes acute, subchronic and chronic experiments. The acute experiment is performed for preliminary evaluation of the degree of toxicity of a substance using the "fish-test" method. The indicator of toxicity is the death of the experimental fish. It is reasonable to conduct the experiment at relatively high temperatures, and relatively low oxygen content, taking into account the range of fluctuations in natural water body. Water hardness and pH value are selected in such a manner as to show the maximum toxicity of the substance tested. The least resistant fish species of ichthyofauna tested should be used as the test object. In this case, it is important to keep in mind the characteristic of resistance for the given species at various stages of ontogenesis, selecting the least resistant from them. Taking into consideration the relation of lethal effect and test duration, the duration of the acute test should be limited to 24 hours.

A subacute test is carried out to show the path of the toxicant's effect on fish and function development mechanisms, with the most sensitive methods for determining the threshold concentration demonstrated in the chronic test. Concentrations of the substance which possess an expressed toxicity effect on the organism are used. They are usually found within a range of 1/2-1/10 of the lethal concentration. The test is conducted on the least reistant species of fish. Duration of the tests is from 10 days to 1 month. Since the toxicodynamics of the majority of substances discharged into water bodies is unknown (applicable to fish), the most complete set of indicators possible which allow us to evaluate the functional condition of the organism's various systems is necessary. Together with indicators integrally reflecting the organism's condition, such as increase in live weight, level of feeding excitability, and intensity of oxygen consumption, finer (or more delicate) physiological and biochemical indicators (activity of various enzymes, hemotological indicators, humoral and cell factors of inborn immunity, behavioral reactions, and electro-physiological tests, which characterize the condition of various functional systems should be used.

The chronic test - is the final stage of ichthyotoxicological research. Its task is to demonstrate the threshold concentration, toxic effect zone and maximum inactive concentration. It is expedient to test 3-5 concentrations with a five-fold interval. The initial concentration and range of concentrations are selected from data obtained in the acute and subacute tests. The duration of the chronic test is from 1 to 3 months, but no longer. To shorten the duration of the tests, it is expedient to use the functional loading method, and on this basis to determine the condition of the indicator function (selectivity imitated), established on the basis of data from the subacute test.

Special emphasis was given to ichthyotoxicological tests when setting biological standards for MPC, since the toxicological indication of harm is more dangerous than all components of the natural ecosystem, and consequently, for the "health" of the water body. However, this does not mean that in biological standard setting, it is always necessary to be ruled solely by this sign. That principle is composed when actually setting hygienic standards, and with whose agreement the MPC of the substance tested is established according to the limiting factor of harm (general sanitary, organoleptic or toxicological). For example, the standard may be established according to the least concentration of the substance which demonstrates an unfavorable influence on the water body. This criteria must be preserved to the end that setting biological standards be fully self justifying.

Considerations of principles and methods for biological standard setting for chemical substances stated in this report, and evaluations of the level of pollution in water bodies require further development.

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SECTION 5

CHRONIC EFFECTS OF LOW LEVELS OF HYDROGEN CYANIDE ON FRESHWATER FISH

Lloyd L. Smith, Jr.

INTRODUCTION

The toxicity of cyanides to fish has long been recognized. Compounds containing the cyanide radical are frequently present in effluents of many industries including electroplating plants, steel mills, petroleum refineries and gas works. In aqueous solution the cyanide radical from simple alkali cyanides such as NaCN hydrolyzes to form free cyanide (CN ion and molecular HCN). The molecular (un-ionized) component predominates at pH values found in most natural waters, with less than 4 percent of free cyanide occurring in the ionic form below pH 8 at 25 °C. As the pH of aqueous simple cyanide solutions is increased, the percentage of free cyanide present as the CN^- ion is increased to satisfy the equilibrium reaction of $HCN \rightleftarrows H^+ + CN^-$.

Little information is available on the long-term effects of hydrogen cyanide on fish. Neil (1957) and Broderius (1970) found that free cyanide concentrations of 10 μg/liter, expressed as CN, impaired the swimming performance of salmonid fishes. Leduc (1966) measured the growth of juvenile cichlids (*Cichlasoma bimaculatum*).

The work reported here was designed to determine the effect of low levels of HCN on the fathead minnow, *Pimephales promelas*, from egg through the juvenile period of the second generation, and on brook trout, *Salvelinus fontenalis*, adults through egg maturation to spawning and development of the second generation to advanced juvenile stage.

MATERIALS AND METHODS

Experiments with fathead minnows were started with eggs from laboratory stock originating at the Duluth laboratory of the U.S. Environmental Protection Agency. The brook trout adults utilized were from the State of Wisconsin hatchery at Osceola, Wisconsin. Water for experiments was taken from the laboratory well (Table 1).

Eighty fathead minnow larvae were placed in each of 15 20-liter glass tanks, and sodium cyanide solution was introduced to the chambers with a

TABLE 1. ANALYSIS OF WELL WATER USED IN BIOASSAYS[a].

Item	Concn (mg/liter)
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Magnesium as $CaCO_3$	70
Iron	0.02
Manganese	0.04
Chloride	<1.0
Sulfate	<5
Fluoride	0.22
Total phosphorus	0.03
Sodium	6
Potassium	2
Ammonia nitrogen	0.20
Organic nitrogen	0.20
Phenols	<0.005
Cu, Cd, Zn, Ni, Pb, Hg	<0.01

[a] Water taken from well head after iron removal and before aeration and heating.

toxicant dispensing system designed by Mount and Warner (1965). This system cycled every 3 min to deliver 1 liter of water and a measured amount of toxicant. Twelve levels of HCN were maintained from 5 to 100 μg/liter at pH 8.06-8.09, 24.8-25.1 °C and dissolved oxygen of 5.9-6.3 mg/liter. Cyanide concentrations were analyzed by the Epstein colorometric method (APHA, 1971) from samples taken in the test chambers 3 times per week. HCN was calculated from dissociation constants of Izatt, *et al.*, (1962). Three controls with well water were run simultaneously with the cyanide treatments.

After 106 days minnows were transferred to 20 treatment and 5 control chambers, each containing 35 liters of water and 20 fish. In 149 days or when first spawning occurred, four mature males and three females were selected and left in each treatment tank.

After 192 days total exposure of fry and spawning adults, 50 eggs from each treatment were placed in plastic cylinders with a screen on one end. Cylinders were suspended in the same test water as adults and oscillated until the eggs hatched. After 227 days from the start of exposure of the parent generation, a growth and survival experiment was started with second-generation fry. Length was determined photographically after 28 days of fry exposure, and length and weight were measured directly at termination after 56 days.

Ten 19-month-old adult brook trout were placed in 340 liter tanks with eight cyanide treatments and two controls. Treatments ranged from 5.7-75.3 μg/liter HCN at 7.95 pH, 9.0-15 °C and 6.5-7.9 mg/liter O_2. The HCN metering apparatus utilized was the same as for fathead minnows. Temperature, pH and cyanide analyses were made 3 times per week. Exposure began on May 5 and continued through spawning 196 days later. After 143 days, exposure spawning boxes were placed in the tanks and the number of fish in each tank reduced to two males and four females. Spawned eggs were removed from each box each day.

From each spawning of 100 viable eggs, 50 were randomly selected and placed in oscillating cups to hatch at 9 °C. Viability was determined at 12 days by development of the neural keel. Twenty-one days after hatch, 25 alevins (larvae) 15-19 mm long, depending on previous treatment, were placed in 20-liter glass tanks where they were held at 9 °C. There were 3 replications in each of 8 treatments ranging from 5.6 to 77.5 μg/liter HCN and 3 controls. Fish were measured photographically at the end of each 30-day period, and at 90 days they were weighed. After 60 days, fish numbers were reduced to 20. Alevins were fed an unrestricted diet of dry trout food in pelletized form.

RESULTS

Fathead - First Generation

After 28 days, survival in the first-generation experiment averaged 64 percent in the 3 controls and ranged from 80 to 11 percent in treatments. Between 28 and 56 days, survival was 80 percent or greater in all chambers. Survival was 95 percent or greater in all chambers between 56 and 84 days (Table 2). Mortality rate in these two periods was not significantly correlated with HCN concentration at the 0.05 level. After 56 days exposure, the mean weight of fish in the treatments ranged from 130 to 65 percent of that in controls, and from 128 to 86 percent of mean weight in controls after 84 days. The length of time from the start of HCN exposure to the onset of spawning averaged 156 days in controls, and ranged from 148 to 206 days in treatment chambers. In no treatments did spawning begin significantly earlier or later than in the controls.

Mean egg production and egg production per female in each chamber are shown in Table 3. Egg production per female in treatments ranged from 72 percent of that in controls to zero (Table 3). Egg production per female was significantly reduced relative to controls in HCN treatments of 19.6 µg/liter and higher. Mean percentage hatch of eggs spawned and incubated in the tests (Table 3) ranged from 83.9 percent in controls to 21.6 percent at 63.6 µg/liter and 0 percent at 80.7 µg/liter (Figure 1). When

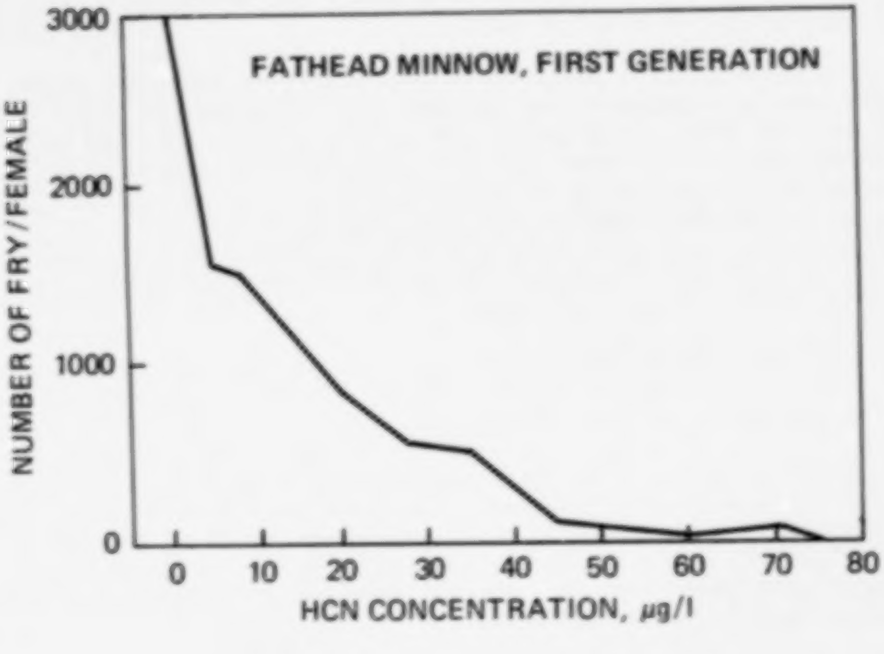

Figure 1. Number of fry produced per 100 grams of female fathead minnow in HCN. Egg production times percentage hatch.

TABLE 2. SURVIVAL AND WEIGHT OF FIRST-GENERATION FATHEAD MINNOW AFTER 28 DAYS, 56 DAYS, AND 84 DAYS OF EXPOSURE TO HYDROGEN CYANIDE*.

Treat-ment	Mean HCN** Concentration (g/l)	Percentage Survival***			Mean Weight (g)	
		0-28 Days	28-56 Days	56-84 Days	56 Days	84 Days
	Control	64	94	100	0.292	0.581
	Control	71	100	100	0.236	0.588
	Control	58	100	100	0.354	0.668
1	5.9	80	100	98	0.205	0.580
2	11.4	59	100	100	0.274	0.676
3	17.9	60	98	95	0.270	0.631
4	24.7	51	100	100	0.296	0.688
5	32.8	46	100	97	0.382	0.785
6	40.5	59	100	100	0.272	0.555
8	57.5	49	97	97	0.190	0.458
9	66.8	49	87	100	0.220	0.528
10	75.3	29	91	100	0.264	0.629
11	88.9	19	80	100	0.199	0.560
12	98.1	11	100	100	0.190	0.627

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*Chambers originally contained 80 larvae. Numbers were reduced to a maximum of 40 per chamber after 56-day measurements.

**84-day period.

***Survival of fish present at beginning of period.

TABLE 3. EGG PRODUCTION, EGG SURVIVAL AND TERMINAL WEIGHTS OF FIRST-GENERATION ADULT FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF CYANIDE.

Treatment HCN (μg/l)	No. Tests	Mean Eggs Per Female	Mean Percentage Hatch	Mean Fry/100 Grams Female	Mean Weight Surviving Adults (g)	
					Male	Female
Control	5	3,476	83.9	2,916	4.42	2.45
5.8	2	2,512	61.6	1,547	4.50	2.60
12.9	2	1,845	81.3	1,500	4.60	2.11
19.6	2	1,468	56.4	828	4.31	2.33
27.3	2	1,367	39.3	537	4.89	2.06
35.8	2	1,010	50.6	511	4.03	2.50
44.2	2	1,119	12.8	143	5.00	2.26
63.6	2	72	21.6	16	4.42	2.63
72.8	2	319	19.6	62	4.21	2.19
80.7	2	242	0.0	0	4.49	2.23
100.7		0	0.0	0	0.0	2.21

the parent experiment was terminated, weights of survivors of either sex in the treatment chambers did not differ significantly from weights of control fish (Table 3).

Fathead F_1 Generation

Survival of fry in the F_1 generation after 28 days was generally higher than in the parent experiment. Survival averaged 84 percent in the three controls and ranged from 88 percent at 26.3 μg/liter HCN to 36 percent at 81.0 μg/liter HCN (Table 4). Mortality rate and HCN concentration were not significant correlated at the 0.05 level. Over the period of 28 to 56 days, all chambers had 81 percent survival or higher. Mean length of fish in treatments after 28 days ranged from 116 to 64 percent of that in controls. The fish at 26.3 μg/liter were significantly longer than control fish, and those exposed to treatment of 34.8 μg/liter and greater were significantly shorter than control fish.

Mean total lengths of fish in treatments after 56 days ranged from 105 to 81 percent of mean length in controls, and mean weights ranged from 122 to 52 percent of that in controls (Table 4). Weights and lengths of fish in treatments from 5.7 to 52.2 μg/liter were not significantly different than controls. Mean lengths and weights of fish at 61.6, 70.5, 95.9 and 105.8 μg/liter were significantly different than controls.

Brook Trout - Adults

Survival and Growth--

Adult brook trout were subjected to various levels of HCN for 196 days and showed no significant mortality or growth differences ($p>0.05$) associated with cyanide concentration. In treatments of 53.9, 64.9 and 75.3 μg/liter HCN, one fish died in each after temperature was reduced to 9 °C. In the two highest treatments fish showed increased irritability when temperature was reduced.

Spawning and Egg Production--

Spawning started in controls and at 5.7 μg/liter HCN 145-147 days after treatment started, but at higher treatments, spawning did not start until 156-159 days after treatment began (Table 5). The number of eggs deposited per 100 grams of female varied from 357 in one control to 106 at 75.3 μg/liter HCN (Figure 2). The number of fertilized eggs per 100 grams of female varied from 293 in one control to none at 64.9 μg/liter HCN. The percentage of live eggs 12 days after hatching varied from 93.6 percent of fertilized eggs in the control to 64.1 percent at 53.9 μg/liter HCN and 0 percent at 64.9 μg/liter. Sperm mobility was tested at 11 HCN concentrations but no significant relationship ($p>0.05$) with HCN was noted.

Egg Survival and Hatch--

Eggs were incubated and alevins held for 90 days at HCN concentrations of 5.6 to 77.2 μg/liter at 9 °C and 64-90 percent saturation of oxygen. No significant differences from controls in percentage hatch was observed (Table 6). Survival of alevins for 90 days after hatching were not signi-

TABLE 4. SURVIVAL, LENGTH, AND WEIGHT OF FATHEAD MINNOW AFTER 28 DAYS AND 56 DAYS OF EXPOSURE TO HYDROGEN CYANIDE[1].

Treatment	HCN Conc. (µg/l)	Percentage Survival[2] 0-28 Days	Percentage Survival[2] 28-56 Days	Mean Total Length (mm) 28 Days	Mean Total Length (mm) 56 Days	Mean Weight After 56 Days (g)
Control A[3]		85	100	15.0	29.2	0.238
Control B[3]		83	98	14.2	28.7	0.238
Control C[3]		84	95	13.6	26.7	0.205
1	5.7	80	100	14.5	28.7	0.234
2	12.2	39	94	14.7	28.0	0.258
3	20.5	60	100	13.9	28.6	0.221
4[4]	26.3	88	95	16.6*	29.6	0.277
5	34.8	48	100	12.5*	27.6	0.184
6[3]	43.0	64	100	12.0*	27.1	0.212
7[3]	52.2	58	98	10.8*	26.8	0.199
8[3]	61.6	64	100	11.7*	26.7*	0.172*
9	70.5	56	95	10.5*	25.9*	0.160*
10[5]	81.0	36	95	10.0*	24.8	0.163
11[3]	95.9	64	100	10.4*	24.8*	0.150*
12[3]	105.8	40	81	9.2*	22.9*	0.188*

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[1] Except where noted, chambers originally contained 80 larvae. Numbers were reduced to a maximum of 40 per chamber after 28-day measurements.

[2] Survival of fish present at beginning of period.

[3] Larvae spawned and hatched in control chambers of parent experiment.

[4] Began with 42 larvae.

[5] Began with 59 larvae.

[6] Values are significantly different from control values according to Dunnett's procedure (two-tailed; $\alpha = 0.05$).

TABLE 5. EGG PRODUCTION OF ADULT BROOK TROUT EXPOSED TO HCN 144 DAYS BEFORE START OF SPAWNING.

	Start of Spawning	Sex Ratio M/F	Total Eggs/100 g of Female		Percentage[1] Viable (12 Days)
			Deposited	Fertilized	
Control	147	2/4	237	128	93.6
Control	145	2/4	357	293	93.4
5.7	145	2/4	299	246	89.9
11.2	159	2/4	162	137	78.1
32.3	157	2/4	208	119	72.9
43.6	156	2/3	189	171	86.6
53.9	157	2/3	187	124	64.1
64.9	N	2/3	122	0	0
75.3	N	2/2	106	0	0

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[1] Formation of neural keel.

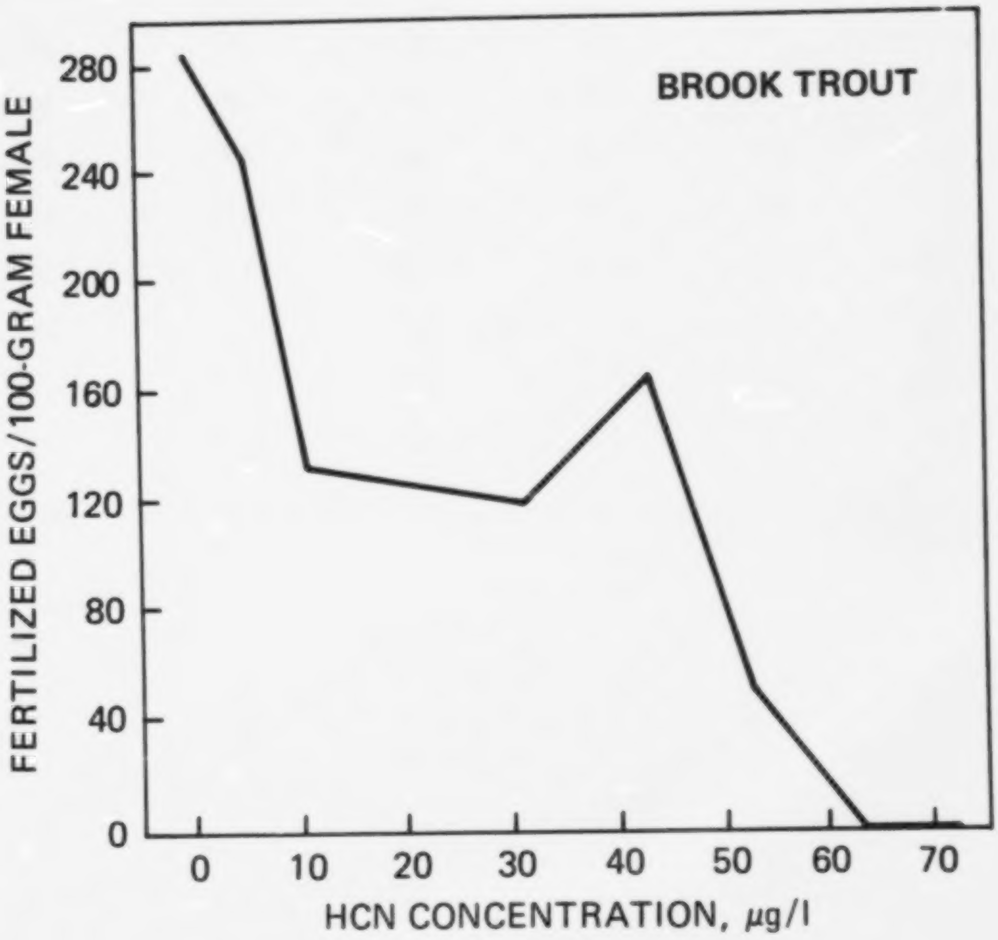

Figure 2. Number of fertilized eggs produced per female brook trout in HCN.

TABLE 6. PERCENTAGE SURVIVAL OF BROOK TROUT EGGS AND ALEVINS EXPOSED TO VARIOUS LEVELS OF HCN.

HCN Conc. (µg/l)	Mean Hatch		Survival of Alevins (90 Days	
	No. Tests	Percentage	No. Tests	Percentage
Control	5	72.5	3	98.6
5.6	4	46.5	2	100.0
11.3	3	70.8	2	100.0
21.8	2	72.0	2	94.0
33.3	3	70.0	2	100.0
43.5	3	72.9	2	100.0
55.3	3	81.3	2	84.0
67.2	2	86.9	2	73.5
77.2	3	76.7	2	30.0

ficantly affected at HCN levels of 43.5 μg/liter and lower. At concentrations of 55.3 to 77.2 μg/liter survival was significantly less than controls ($p \leq 0.05$). At 77.2 μg/liter survival from hatch was 30.0 percent compared to 98.6-100 percent in controls.

Growth of Brook Trout Alevins--

Length and weight of alevins at hatch was not significantly different at the various HCN concentrations, but growth over a 90-day period was markedly affected by increased HCN concentrations (Table 7). The effect on growth was noted after 30 days and was great at 90 days. Fish at all treatment levels from 33.3 to 77.2 μg/liter were significantly shorter and lighter than controls (Figure 3). At 90 days length varied from 41.9 mm in controls to 24.3 mm at 77.2 μg/liter. Weight varied from 1.03 g in controls to 0.16 g at 77.2 μg/liter. At the highest concentration weight was 15.7 percent of controls. At 11.3 μg/liter growth after 90 days was significantly greater than the control, but at 5.6 and 21.8 μg/liter no significant difference from controls was noted. All fish at treatment levels from 33.3 to 77.2 μg/liter at 90 days were significantly slower in growth than controls.

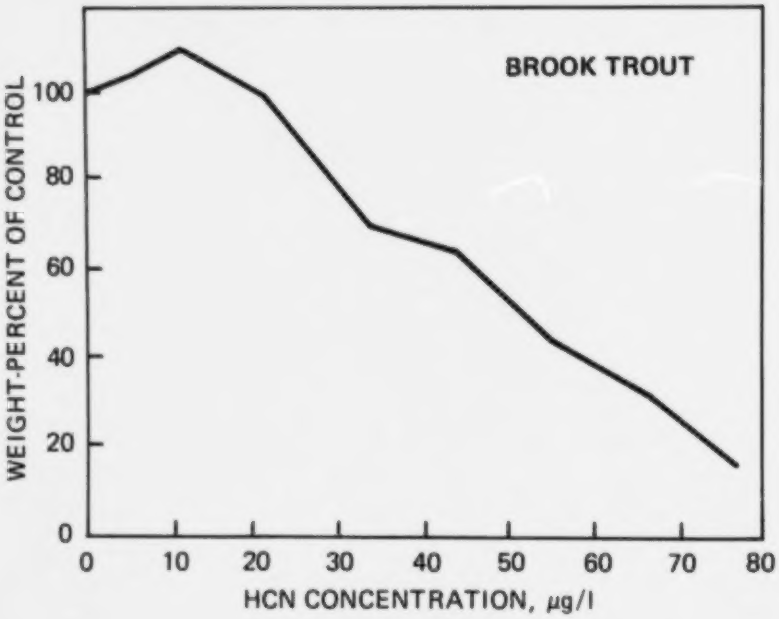

Figure 3. Growth of brook trout in various concentrations of HCN. Expressed as percentage of controls.

TABLE 7. GROWTH OF BROOK TROUT ALEVINS FROM HATCH TO 90 DAYS EXPOSED TO VARIOUS LEVELS OF HCN.

HCN (μg/l)	No. Tests	Mean Length (mm)		Mean Weight (g)		
		At Hatch	90 Days	At Hatch	At 90 Days	% of Control
Control	3	13.3	41.1	40.0	1.030	100.0
5.6	2	13.6	41.9	40.8	1.087	105.0
11.3	2	13.4	43.3*	40.3	1.135*	110.0
21.8	2	12.9	42.0	40.0	1.020	99.0
33.3	2	12.2	37.3*	38.0	0.722*	70.1
43.5	2	13.1	36.0*	40.0	0.660*	64.1
55.3	2	12.3	32.6*	39.7	0.469*	45.5
67.2	2	11.8	30.2*	41.0	0.335*	32.5
77.2	2	11.9	24.3*	39.7	0.162*	15.7

*Significantly different than controls.

DISCUSSION

Most mortality among fathead minnows occurred in the first 28-day exposure to HCN in both parent and F_1 generations (Table 4). The number of eggs produced and fry which survived were reduced at 196 μg/liter and at higher concentrations. It is estimated that the highest "no-effect" level of HCN is between 12.9 and 19.6 μg/liter based on egg production. The lethal threshold for juvenile fathead minnows (defined as the HCN concentration at which no fish die for 48 hours after continuous exposure for 96 hours or longer) as determined by unpublished data from our laboratory is 119 μg/liter HCN at 25 °C, pH 8.0 and 6.0 mg/liter DO (Table 8). Comparison of this acute toxic level to the "no-effect" level indicates that the safe level for fish is between 11 and 16 percent of the acute toxicity concentration of HCN.

When adult brook trout, prior to spawning, were exposed to HCN, at all concentrations greater than 5.7 μg/liter, a reduction in the production of fertilized eggs occurred. When spawning was successful, egg viability was not affected adversely at 43.6 μg/liter and lower. Growth rate of juvenile brook trout during the first 90 days after hatching was reduced at concentrations of 33.3 μg/liter HCN and higher (Figure 3). At 77.2 μg/liter it was 15.7 percent of controls. On the basis of acute threshold toxic levels of 88 μg/liter at 10 °C (unpublished laboratory data) for juvenile fish and 5.7 μg/liter HCN as a safe concentration for successful spawning, the "no-effect" level is approximately 7 percent of the acute toxic level.

From these chronic exposure tests of two fish species, it is evident that safe levels of HCN in the environment are much lower than the concentrations which will kill fish on short exposure. Where there is continuous exposure to low levels of cyanide from steel mills or other sources of cyanide, some fish populations can be adversely affected by concentrations higher than 7-12 μg/liter.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the contributions of David Lind to the experiment of fathead minnows, and to Walter Koenst for the experiment on the brook trout.

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TABLE 8. NINETY-SIX HOUR LC_{50} AND THRESHOLD CONCENTRATIONS OF HCN TO FATHEAD MINNOWS AND BROOK TROUT JUVENILES (μg/liter).

No. Tests	O_2 (mg/l)	°C	LC_{50}	
			96 h	Threshold
		Fathead Minnow		
6	6.0	15	108	108
6	7.0	20	107	107
2	6.0	25	119	119
		Brook Trout		
4	6.0	7	66	65
2	8.8	10	89	88
6	8.0	10	82	75

[1] pH 8.0.

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SECTION 6

MICROBIOLOGICAL INDICES OF THE QUALITY OF WATER AND METHODS OF THEIR DETERMINATION

V.I. Romanenko

In the majority of cases, microbiological indices may be the best way to characterize the quality of water used for both drinking and industrial purposes. Microorganisms are excellent indicators which often exceed the sensitivity of chemical and physical methods. The cells of microorganisms react to minute changes in external medium. Under favorable conditions they start to multiply more rapidly and their metabolism accelerates. Some species of bacteria can exist only in the presence of a definite class of chemical substances. Exhaustive knowledge is not available on the life of millions of microorganisms, however, in the future, the expanded use of microbiological indicators will undoubtedly develop.

It should be recognized that some of the major questions can be correctly answered only by microbiological specialists who are well versed in the details of microbiological technique. In some cases it may be necessary for the knowledgeable scientist to intentionally depart from established methodology, while a similar departure in the hands of a neophyte may lead to false results. When working with pathogenic microorganisms is considered, this may lead to serious consequences.

The present communication deals primarily with bacteria. Algae and small invertebrates, including abundance, or activity, intensity of photosynthesis, while important as excellent indices of the condition of a water-body, are not included in this consideration.

Microbiological indices may be divided into two categories as: (1) the presence of bacteria, and (2) the intensity of one or another bacterial process (Figure 1).

DETERMINATION OF THE QUALITY OF WATER BY THE NUMBER OF BACTERIA OR PRESENCE OF PARTICULAR SPECIES

Quantity of Microorganisms

The quantity of microorganisms may be judged by their total content, or by the content of separate physiological groups. Total numbers of bacteria may characterize the condition of the water-body in general,

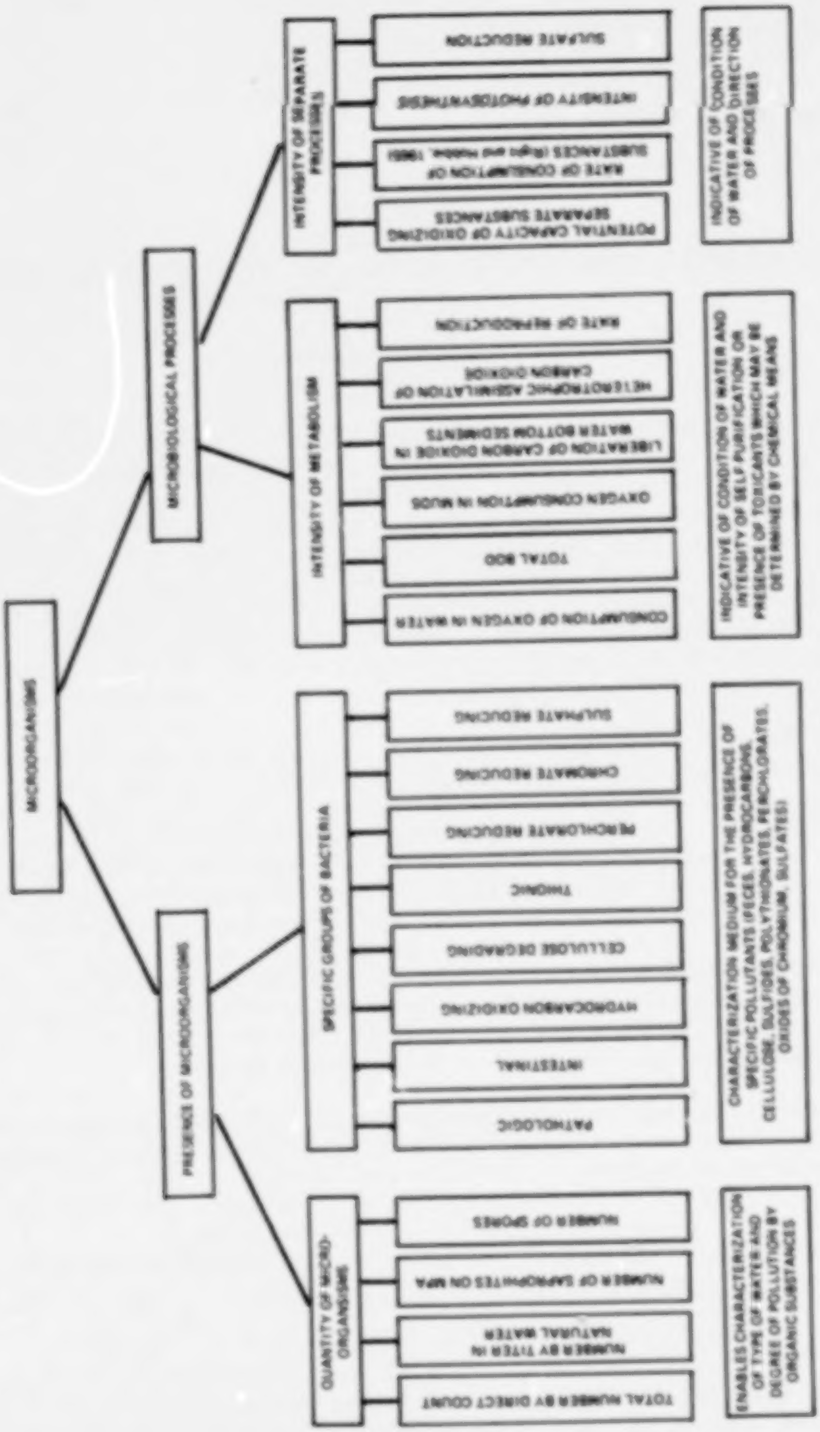
MICROORGANISMS
MICROBIOLOGICAL PROCESSES
PRESENCE OF MICROORGANISMS
INTENSITY OF SEPARATE PROCESSES
INTENSITY OF METABOLISM
SPECIFIC GROUPS OF BACTERIA
QUANTITY OF MICRO-ORGANISMS
SULFATE REDUCTION
INTENSITY OF PHOTOSYNTHESIS
RATE OF CONSUMPTION OF SUBSTANCES (Wright and Hobbie, 1966)
POTENTIAL CAPACITY OF OXIDIZING SEPARATE SUBSTANCES
RATE OF REPRODUCTION
HETEROTROPHIC ASSIMILATION OF CARBON DIOXIDE
LIBERATION OF CARBON DIOXIDE IN WATER BOTTOM SEDIMENTS
OXYGEN CONSUMPTION IN MUDS
TOTAL BOD
CONSUMPTION OF OXYGEN IN WATER
SULPHATE REDUCING
CHROMATE REDUCING
PERCHLORATE REDUCING
THIONIC
CELLULOSE DEGRADING
HYDROCARBON OXIDIZING
INTESTINAL
PATHOLOGIC
NUMBER OF SPORES
NUMBER OF SAPROPHITES ON MPA
NUMBER BY TITER IN NATURAL WATER
TOTAL NUMBER BY DIRECT COUNT
INDICATIVE OF CONDITION OF WATER AND DIRECTION OF PROCESSES
INDICATIVE OF CONDITION OF WATER AND INTENSITY OF SELF PURIFICATION OR PRESENCE OF TOXICANTS WHICH MAY BE DETERMINED BY CHEMICAL MEANS
CHARACTERIZATION MEDIUM FOR THE PRESENCE OF SPECIFIC POLLUTANTS (FECES, HYDROCARBONS, CELLULOSE, SULFIDES, POLYTHIONATES, PERCHLORATES, OXIDES OF CHROMIUM, SULFATES)
ENABLES CHARACTERIZATION OF TYPE OF WATER AND DEGREE OF POLLUTION BY ORGANIC SUBSTANCES

Figure 1. The scheme of microbiological indices of water quality.

i.e., the type of water-body. Through the efforts of Soviet Scientists, the total number of bacteria has been investigated in detail in waters of differing type. These investigations may, in general outline, be represented as in Table 1.

TABLE 1. CONTENTS OF BACTERIA IN WATERS OF VARYING TROPHIC DEGREE.

Type of Water-Body	Quantity of Bacteria mln/ml	Water-Body
Oligotrophic	0.1-0.5	Lakes Onega, Baikal
Mesotrophic	0.5-1.5	Rybinskoe Reservoir
Eutrophic	1.5-10	Tsymlyanskoe, Kakhovskoe Reservoirs
Distrophic	1.5-3	Lake Melnezers in Latvia

In water-bodies of the oligotrophic type with clean water, the number of bacteria varies from 0.1 to 0.5 mln/ml. With an increase in the trophic degree, the number of bacteria also increases. In mesotrophic water bodies the number reaches 0.5-1.5 mln/ml, and in eutrophic waters, 1.5 to 10 mln/ml. Distrophic waters are distinguished by high water color values. The content of bacteria in them are the similar to the mesotrophic condition, but the activity of these forms is considerably reduced.

The methodology associated with determination of the total number of bacteria in water appeared as a result of the development of the ideas of Vinogradski (1952) on the content of bacteria in soils. There are several varieties of estimated strengths of bacteria in water (Kuznetsov and Karzinkin, 1930). The most suitable of these methodologies, used at the present time by most research workers, is the one suggested by Razumov (1932).

For calculation of bacterial numbers, 1 to 50 ml of water, depending on the trophic degree of the water body, is filtered through a membrane filter with a pore size 0.2-0.3 mm. The filters are dried, stained with laboratory conditioned erythrosine and the cell production is counted under the immersion microscope. Calculations are made with due consideration of the volume of filtered water (Rodina, 1965).

Determination of Living Bacteria by the Method of Titer in Sterile Water Using C-Hydrolysate of Protein

A distinguishing feature of many microorganisms is the fact that they do not develop on classic nutrient media (meat-peptone agar, meat-peptone gelatine, etc.). As has been demonstrated (Romanenko, 1973), they grow well on media with minimum quantity of organic matter, equivalent by composition to that in natural water.

Determination of the number of living bacteria should involve the use of water from the investigated water-body. The water should be collected in bottles, then decanted into 10 ml test tubes and sterilized in an autoclave.

Since sterilization partially destroys the carbonates making the water more alkaline, following autoclaving, the test tubes should be placed into an atmosphere of carbon dioxide rendering the water neutral. The test-tubes are subsequently placed in a stand in the order shown in Figure 2. To the first test tube is added 1 ml of water by sterile pippette. After thorough mixing, 1 ml of its contents is transferred into the second test-tube. The process is repeated until the third test tube is reached where subsequent transfers are performed in three replicates for a greater statistical confidence. For majority of water bodies, 6-7 dilutions should be made. The seventh or the eighth test-tube serves as control. Then the test tubes are placed for 7 days in a thermostati-

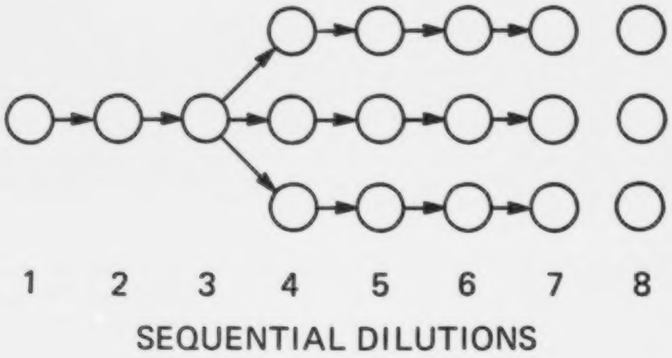

Figure 2. Scheme of the order of test tubes in determination of living bacteria by titer method. Numbers represent the sequence of dilutions.

cally controlled incubator at a preselected temperature, usually 26 °C. One drop of the solution of ^{14}C-labeled protein hydrolysate having a measured activity under a Geiger counter of the order of 0.2×10^6 imp/min is added to each of the test-tubes by means of a Pasteur pippette, and the test-tubes are incubated for 2 hours. Their contents are then fixed with 0.25 ml of formalin and filtered through membrane filters with a pore size 0.2-0.3 mm. After fixation and filtering, 5 ml of physiological solution is filtered to remove the excess portions of the labeled preparation. The filters are dried and the radioactivity of bacteria is measured under the Geiger counter. The final dilution in which the radioactivity differs markedly from the control serves as an indicator of the limit of dilution for bacterial reproduction. Using this methodology and various classes of radioactive substances (e.g., phenol) the presence of microorganisms and their number in the water samples may be determined.

Number of Saprophytic Bacteria

The number of saprophytic bacteria is the most reliable and sensitive indicator of water pollution by organic substances of household origin. This is a classic method used for about 100 years by sanitary organizations. It was proposed by Koch and has been used for counting of bacteria in water by a number of different workers. The method of determination of the number of saprophitic bacteria is quite simple. In the USSR, standard dry nutrient medium (FPA) is made from fish flesh to which peptone, sodium chloride and 15 percent agar-agar are added.

The medium to be inoculated is prepared in 50-100, or 200 ml flasks depending on the quantity required. The water is taken into sterile glassware by special samplers. The most simple model is the sampler of Meier-Frantsev (Romanenko and Kuznetsov, 1974). Inoculation must be made within one half of an hour after sampling. Samples may be stored in refrigerator or vacuum flask at low temperature for no longer than a day.

Inoculation may be of either the surface or depth type. In the former case, the FPA should be melted. In laboratory this is best done in boiling water. The flask with FPA is placed into boiling water until all the medium is molten (not even smallest lumps of solid medium must be left, otherwise the analysis will be spoiled). The medium may also be melted on an open flame of a burner or on electric plate, but the procedure must be carefully watched. The medium is then cooled to 40 °C. In practice, microbiologists apply the flask to the cheek, if the medium does not burn, it may be poured into Petri dishes.

For deep inoculation, the water to be tested is added into sterile Petri dishes by sterile pippettes, then the FPA is poured and all is thoroughly mixed. In this case, bacteria grow throughout the medium. The colonies grown in the depths are physically smaller in size. For surface inoculations, the medium is poured into the dishes, and after it solidifies 0.5 to 1 ml of inoculum is added upon the surface and spread over with a help of sterile glass spatula. The dishes are incubated at a room temperature for 10 days. Then the number of colonies are counted, and estimations are made with due consideration to the dilution.

A good indicator of the cleanliness of water is the ratio of the number of saprophytic bacteria to their total number expressed in percent (Kuznetsov, 1952; Romanenko, 1971). A summary is presented in Table 2.

TABLE 2. RATIO OF THE NUMBER OF SAPROPHYTIC BACTERIA TO THEIR TOTAL NUMBER AS AN INDEX OF THE CLEANLINESS OF WATER.

Ratio, %	Water	Water-Body
0.003 or less	Very clean	Lakes Onega, Ladoga, Baikal
0.03	Clean	Reservoirs: Rybinskoe, Sheksninskoe Ruibyshevskoe
0.3	Dirty	Some parts of Volga River
3 or greater	Very dirty	Collectors of waste waters

Water in which the ratio is 0.003 percent may be considered exceedingly clean; a ratio of 0.03 suggests clean conditions; 0.3, dirty; and 1-3 and higher, very dirty.

Content of Bacterial Spores in Waters

Bacterial populations in water are dominated by non-spore forming bacteria. The ratio of bacillary forms to other bacterial groups is frequently equal to 1:10.

In view of some workers, the spore forming bacteria are more often found in the presence of hard to degrade organic substances, e.g., humic compounds, etc. (Kholodnyi, 1957). In fact, spore forming bacteria are more abundant in waters colored by humic substances, and in drainage from peaty grounds. The number of spores is increased in proportion to the vegetative cells.

Determination of the total number of spore forming bacteria is laborious and a time consuming procedure. The water or sediment to be tested is inoculated onto agar plates according to the methods of Koch. It is then necessary to wait for some time until the colonies age and begin to produce spores, a microscopic examination of the colonies is performed using preferential staining of the spores (Omelyanski, 1932).

It is easier to determine the presence of spores in water than it is to enumerate the spore-forming bacteria. For this type of determination, two methods may be used. Both are based on the destruction of the vegetative cells, and subsequent creation of conditions favorable for germination of the spores.

Method of Heating--

A sample of water or sediment, either directly or after dilution, is heated in a water-bath for 10 min. at a temperature of 80 C. Test-tubes with tested water are then cooled and inoculation is made on MPA, or a mixture of MPA and yeast-agar. The number of colonies is counted after incubation.

Treatment of Samples with 96 Percent Ethyl Alcohol

Romanenko and Daukshta (1975) have shown that the vegetative cells of microorganisms die almost instantly under the action of strong ethyl alcohol, but the spores are preserved for a considerable period of time. This is the basis for the second method of determining the quantity of spores in a sample of water or mud.

The spores can be separated from the vegetative cell by several methods. The tested water may be mixed in a test tube with alcohol and then inoculated. To 5 ml of test water, 10 ml of ethyl alcohol is added (ratio 1:3 in any volume), and 0.5 ml of the mixture is inoculated into Petri dishes with MPA by the depth technique (with or without dilution using sterile water). Another method is to pass a known volume of the test water through a membrane filter, then 3-5 ml of ethyl alcohol is passed through the filter followed by 3 ml of sterile distilled water to wash the alcohol from the filter. The filters are then placed upon a layer of agar in Petri dishes. The number of spores is estimated after incubation by the number of colonies present. This method is particularly good in the case of clean water, where the number of spores is small, because of the concentrating effect of filtering.

The number of spores in mud deposits or sediments, can be determined by yet another method. A row of test tubes with sterile water for dilution is used. One test tube, for example N 3 or 4, is filled with 9 ml alcohol (see Figure 3). Thus, the water is passed through the alcohol in the process of

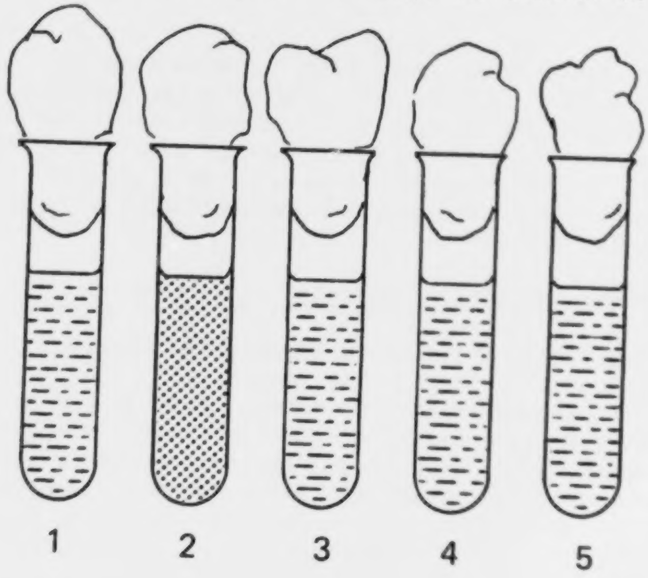

Figure 3. Position of the test-tube (N 2) with ethyl alcohol in the row when determining the spores of bacteria in mud deposits.

dilution allowing only living spores to pass the subsequent test-tubes from which water is inoculated onto MPA.

The Number Specific Bacteria

Consideration of the field of medical microbiology is beyond the scope of this paper. It should only be indicated that highly dangerous microorganisms causing infectious diseases may be isolated using special media and methods of enrichment. Isolation of such bacteria indicates a great danger of such water for the health of people. Such water is quarantined, and specific measures are to taken to protect the health of the public. Fortunately such microorganisms are rarely present in water. Usually they enter the water as a result of illness or from carriers. Some species of bacteria may be indicators of pollution of the water by a certain type of organic substances. For example, species of the filamentous bacteria of the genus *Cladothrix* are indicative of the presence of nitrogen-free organic compounds in water, and the presence of species of the genus *Sphaerotilus* indicates to pollution of the water by complex organic compounds of nitrogen (Rasumov, 1961).

Coliform Titer

The presence of coliform bacilli in water is indicative of a fresh pollution with feces. In the water, high concentrations of these bacilli, which are normally inhabitants of the human intestine, indicate pollution, and the possible presence of pathohenic bacteria. Though certain strains of coliform bacteria may persist for long periods of time and even multiply in water, fresh fecal pollution is detected by those strains which only recently entered the water. These are discerned by a characteristic coloration of the colonies.

The number of these bacteria in water is determined by inoculation with the highly specific Endo medium. The colonies can be grown either on this medium directly in Petri dishes, or on membrane filters. In the latter case, the water is passed through the filter, and it in turn, is placed on Endo agar. Gold-colored colonies with a characteristic metallic lustre are counted. The coliform titer is taken as the quantity of water per one coliform-bacillus.

Separate Physiological Groups or Separate Characteristic Bacteria

The presence of a certain class of mineral or organic compounds in water bodies may be established by the presence or increased content of specific groups of bacteria. In many cases, it is easier to detect the presence of such bacteria than it is to establish the presence of certain chemical substances. It is generally accepted that it is possible to isolate separate microorganisms responsible for specific processes. An indication of the presence of that process is demonstrated by detection of an increased content of a certain species or genera of bacteria.

Presence of Liquid Hydrocarbons (Oil Pollution) In Water--

Pollution of water or bottom sediments can be estimated by an increased content of hydrocarbon oxidizing bacteria. For this process the samples of water must be inoculated onto the nutrient medium of Tauson, into which a sterile solution (sterilization is carried out in sealed ampules by repeated boiling) of diesel oil, kerosine, or drop of mineral oil is added as the main source of carbon. After adjusting of pH to the neutral value, the medium is poured into test tubes. Into each, hydrocarbon and dilutions of the tested water are added. The test tubes are placed into an incubator at 26 °C for 5-10 days. At that time, the dilution may be determined in which the film of bacteria was formed or in which the medium became cloudy. In clean water the bacteria will either fail to grow, or will develop in the 1st or 2nd dilution, while in polluted waters they will grow in the 3rd - 5th, sometimes in the 6-7th dilution.

In some cases instead of titer, a time consuming process, the intensity of bacterial development may be determined. For this, the medium is poured into 3-5 ml serum bottles and to each a drop of hydrocarbon and 0.1 ml of the suspect water are added. The bottles are then incubated. In 5-10 days they are examined, and the intensity of bacterial development (formation of the film, turbidity) is noted. The water is considered to be clean if negative, or poor development is observed. When the pollution of the water by petroleum products is significant, a thick skin-like film with a white or pink tint rapidly develops, and occasionally the medium becomes turbid.

Content of Cellulose Degrading Aerobic Bacteria (Pollution of Water With Cellulose--

At the present time, many wood processing plants discharge wastes into natural waters. Often wood fibers, lignin, and the like are found in them. As a rule, cellulose degrading bacteria develop in enormous quantities in the places of accumulation of wood fibers. They may isolated by inoculating specific nutrient media, e.g., medium of Hatchinson (Romanenko, 1971). This medium contains the principal mineral salts required with cellulose (filter paper) as the sole carbon source, with this medium, the inoculum must be used from the 1st to the 6th dilution. Development of bacteria in the 1-2nd dilution indicates the presence of a relatively small number, while development in subsequent dilutions shows the presence of pollution with cellulose.

Presence of Sulphides and Thiosulphates--The presence in water of reduced compounds of sulphur may be estimated by the presence of thionic bacteria. These may be grown on the liquid or solid phase medium of Beiering. The colonies often have a milky coloration owing to liberated sulphur. Colonies can be quantified with a help of autoradiography (Romanenko, *et al.*, 1975). This method utilizes ^{14}C-carbonate added to the agar medium. Bacteria are grown on membrane filters. After incubation, the filters are removed, treated with a weak solution of hydrochloric acid, dried and glued to strip of compact paper equal in size to photographic film. In a light-free environment, the paper is applied to the film, and both are rolled. In 2-5 days the film is developed in a contrast developer. Colonies of thionic bacteria are counted as dark spots on the film.

Presence of Perchlorates and Chlorates--In some cases plants and factories discharge chlorates and perchlorates into receiving waters, e.g., NH_4ClO_3 or NH_4ClO_4. Recently a group of bacteria have been discovered which quickly reduce this compound under natural conditions (Romanenko, *et al.*, 1976). It also has been established that in the bottom sediments of the majority of waters, the processes of reduction of perchlorates are either slow or absent, but in the waters containing wastes of a given industry, the number of the specific groups of bacteria greatly increases.

The activity of these bacteria can be assayed using chlorine labeled perchlorate ($NH_4\ {}^{36}ClO_4$). Nutrient medium is prepared as described elsewhere (Romanenko, *et al.*, 1976). The media consists of salts, microelements, vitamine B_{12}, acetate, meat-peptone broth and perchlorate, 100 mg/l.

The medium is poured into bottles with ground stoppers 60-70 ml in volume. To this is added 5 ml of test water, or 100 mg of sediment, and 1 ml of a sterile solution of labeled perchlorate with activity of 0.1×10^6 imp/min. After 3-5 days the contents of the bottle are filtered through a filter paper made slight acidic with nitric acid. A one percent solution of silver nitrate is introduced which precipitates the chlorides. If the natural content of chlorides in the medium is not great, 2-3 mg of sodium chloride should be added to precipitate the labeled ${}^{36}Cl$ ions. The precipitated chlorides should be filtered through a membrane filter washed with distilled water (10 ml), dried; and the radioactivity of the precipitate $Ag\ {}^{36}Cl$ measured. Accounting for the initial amount of perchlorate added to the medium, one may estimate the amount which was converted to chlorides. If the values are close to zero, the given bacteria may be considered to be absent, and the process of reduction lacking. If reduction occurs, perchlorates may be reduced to the extent of 50-100 percent of the added substance. Control experiments with the samples fixed by formalin should always be conducted.

Presence of Chromates and Bichromates in Water--Chromates and bichromates enter the waters from residues of electroplating shops, automobile factories, or chemical plants. Chromium is heavy metal toxic to many organisms. In 1973 bacteria were isolated which decompose chromates and bichromates under anaerobic conditions to chromium hydroxide using these compounds as oxygen donors (Romanenko and Korenkov, 1975). These bacteria may be used for purification of industrial wastes from chromates and perchlorates (see above), as well as indicators of chromium oxides in wastes.

In the places of permanent discharge of chromates, the water in the near-bottom layers and the surface layer of sediments are rich in chromium reducing bacteria which can be detected by a special inoculating (Romanenko and Korenkov, 1975). The medium is prepared in flasks, sterilized and adjusted to circum neutral pH. It is then decanted into stoppered test-tubes. After inoculations, with the test water, the cultures are incubated for 7-10 days. The presence of the chromium reducing bacteria is indicated by the medium turning from yellow (hexavalent chromium) to colorless (trivalent chromium). Changes in color may be accurately measured on a spectrophotometer.

The presence of chromium reducing bacteria is indicative of pollution of water by chromates. This is also true in the case where hexavalent chromium is absent (indicative of decomposition). In this instance it is impossible to analyse for the presence of chromium with a spectrophotometer.

EVALUATION OF THE QUALITY OF WATER BY THE INTENSITY OF BACTERIAL PROCESSES

Daily Oxygen Consumption for Respiration of Bacteria as an Index of the Water Quality

All the organisms inhabiting water constantly consume oxygen for their respiration. Only in rare instances will oxygen consumption by algae or zooplankton exceed that of bacteria. In most cases, the role of microorganisms in oxygen consumption and, therefore, in destruction of organic matter, is greater than that of all other aquatic organisms taken together. This is demonstrated by the indirect estimations of the number and rate of reproduction of separate groups of organisms. In all cases, bacteria reproduce most rapidly, as a further illustration, in the water passed through a membrane filter where only bacteria remain, the rate of oxygen consumption essentially does not change (Romanenko and Dobrynin, 1973).

It is well established that respiration is biochemical process in which oxygen is combined with organic substances. In the end, the process may be expressed by the following equation:

$$CH_2O + O_2 = CO_2 + H_2O$$

It follows from this that 32 weight parts of molecular oxygen are used per 12 weight parts of carbon of organic matter. Thus, to determine the amount of decomposed organic matter, the number of milligrams of consumed oxygen by is multiplied by 0.375 (i.e., ratio 12:32). This application can be made if the coefficient of respiration is 1, i.e., when carbohydrates are destroyed in respiration. Occasionally 0.8 or 0.9 is used instead of 1. In this case, the value of the accepted coefficient should be multiplied by 0.375. It should be noted that the experimental data on respiration coefficients are very few, and often depart from theoretical consideration.

To determine destruction of organic matter (Vinberg, 1934), 100-150 ml bottles are filled with water straight from the Ruttner sampler. The procedure is performed in such a way so that the water will have as little contact with atmosphere as possible. A rubber tube from the sampler is placed in the bottom of the bottle and approximately 2-3 volumes are passed through it. The bottles are closed with ground glass stoppers. In two bottles, oxygen is measured immediately by the Winkler method (Alekin, 1954). The remaining two bottles are placed in a light-proof bag and incubated for a standard time: a day in oligotrophic and mesotrophic waters, 12 hours in more rich waters, 6-12 hours in eutrophic waters in summer. In winter and summer at low temperatures, the time of incubation may be decreased to several days. The time utilized should be the minimum for which a confident difference is established between the initial and final oxygen content in bottles. The sensitivity of the oxygen methods conducted by a skillful specialist may reach 0.05 mg O_2/l.

Destruction of organic matter expressed in terms of oxygen is equal to:

$$O_{\text{И}} - O_{k} = O_{\Pi} \text{ mg/l/day}$$

where:

O_{H} - initial oxygen content (mg/l),
O_{k} - final oxygen content,
O_{Π} - oxygen consumption.

Approximate summer values for respiration of the aquatic ecosystems in water-bodies of various trophic degree are indicated in Table 3.

TABLE 3. OXYGEN CONSUMPTION FOR RESPIRATION OF ORGANISMS IN WATER-BODIES OF VARIOUS TYPES (SUMMER VALUES).

Type of Water-Body	Approximate Values of Oxygen Consumption, mg O_2/l/day	Quality of Water
Oligotrophic	0.05-0.1	Clean
Mesotrophic	0.1-0.3	Good
Eutrophic	0.3-3	Bad
With organic pollution	3-10	Very Bad

Total BOD

In sanitary microbiology, instead of daily oxygen consumption, the value of total BOD, i.e., quantity of oxygen which may be used by the microflora for oxidation of all the fractions of easily degradable organic matter, is often used (Lapshin, 1952). The experiments are performed at a constant temperature of 20 °C.

Water from the bottom sampler is poured into a flask, its temperature is adjusted to 20 °C, and filled with a syphon into 6 100-150 ml bottles. In a case of low oxygen content, the water is saturated with oxygen by bubbling air through it. In two bottles, the initial content of oxygen is measured by Winkler method, the remaining two are analyzed for BOD. Bottles to be incubated are placed in water and ground covers with the tested water (water lock) are fixed on them.

The difference in oxygen content between the initial water and at the amount present after 3 days is designated as BOD_3, and after 6 days as BOD_6. The total BOD is determined by the formula:

$$BOD_{tot.} = \frac{a_1^2}{2a_1 - a_2}$$

a_1 = BOD for 3 days,
a_2 = BOD for 6 days.

Heterotrophic Assimilation of CO_2

Heterotrophic assimilation of CO_2 is assimilition of carbon dioxide by heterotrophic organisms. As early as 1921, it was shown by A.F. Lebedev and later by Wood and Werkmann (1936) that heterotrophic organisms assimilate a small amount of CO_2 in their metabolism.

Until the present time, this phenomenon was described only in the experiments performed at the Institute of Biology of Inland Waters, Academy of Sciences, USSR (Romanenko, 1964; Sorokin, 1964). By this work it has been shown that a certain amount of carbon dioxide is assimilated per given increase in biomass of bacteria. It has also been found that there is a direct proportionality between assimilation of CO_2 and respiration. As a result, it has been established that the ratios between oxygen consumption, increase of bacterial biomass, and assimilation of CO_2 is equal to 1000:100:7 (mg O_2, mg C, mg C). It should be realized, of course, that this correlation is not as strict as may be found in chemistry and physics. However, it may be used for the determination of productivity of bacterial biomass (Kutnetsov *et al.*, 1966). With the exception of meromictic lakes in the zones above the layer of hydrogen sulphide, heterotrophic assimilation of CO_2 prevails over chemosynthesis in water bodies. Heterotrophic assimilation of CO_2 in waters of varying types in shown in Table 4.

TABLE 4. MEAN MID-SUMMER VALUES OF HETEROTROPHIC ASSIMILATION OF CO_2 IN WATER BODIES OF VARIOUS TYPES

Type of Water-Body	Assimilation of CO_2, g C/l/day	Character of Water
Oligotrophic	0.1-1.0	Very Clean
Mesotrophic	1-5	Clean
Eutrophic	5-70	Dirty
-	10-200	Very Dirty

The high sensitivity of the radioactive carbon method enables the determination of the smallest values of CO_2 assimilation. The method is as follows. 50-70 ml of water is placed in a bottle to which 1 ml of ^{14}C-labeled carbonate, $NaH^{14}CO_3$, is added having a specific activity of 1-5 x 10^6 imp/min under the Geiger counter. The bottles are placed in lightproof bags, and the samples are incubated at the temperature of the water-body for 24 hrs in oligotrophic and mesotrophic waters, 6-12 hrs may be used in eutrophic waters. The samples are then fixed with formalin (0.5 ml per 100 ml of water) and passed through filters impermeable to bacteria. In the laboratory, the filters are placed for 10 min. upon a filter paper moistened with 1 percent solution of hydrochloric acid, dried and counted.

The content of hydrocarbonates should always be measured. This analysis can be performed by direct titration when the water is clean and transparent, and with distillation when it is highly sedimented or polluted. In the former case, 100 ml of water is poured into a conical flask and 3 drops of phenolphtalein solution is added. If the water does not turn pink, 1-2 drops of a solution of an alkali are added. The color is then neutralized by addition of 0.1 N solution of HCl. After the color has disappeared (pH 8.3), 7 drops of methyl red or methyl orange are added, and titration is repeated with HCl until a stable pink color appears. The amount of the acid used for titration from the time of addition of the second indicator is multiplied by 12, thus obtaining the quantity in mg C of carbonate in 1 l of the water.

If the test water is dirty, the carbonates are determined by distillation from acidified solution into an alkali (Kuznetsov and Romanenko, 1963).

The quantity of carbon dioxide assimilated by microorganisms is calculated by the formula:

$$\Gamma a = \frac{r \cdot C_k}{R}$$

where:

Γa - heterotrophic assimilation of CO_2 (μg C 1/day),
r - radioactivity of microorganisms in the whole sample of tested water (imp/min),
C_k - content of carbonates in water (μg C/1),
R - radioactivity of the carbonate solution, added into the sample, imp/min.

Heterotrophic Assimilation as an Index of Bacterial Development

Heterotrophic assimilation of carbon dioxide as an index of bacterial development was first used by the author (Romanenko, 1964). It is based on the proportionality between increase of bacterial biomass and CO_2 assimilation. For this purpose ^{14}C-labeled organic substances may also be used, but here difficulties arise owing to the fact that organic substances quickly decompose, and it is not always possible to establish the location of the experiment on the non-linear assimilation curve. Further rationale favoring the use of carbonates is the fact that organic substances are not introduced with carbonates. The carbonates are essentially neutral substances and in nutrient media there is almost always an excess of carbonate as a buffer. It is not desirable to create an excess of organic substances in nutrient media.

The influence of different substances upon microorganisms may be determined using both pure and mixed cultures. One may also study the effect on bacteria of various substances, for example, heavy metals, antibiotics, antiseptics, pesticides, or toxic solutions of waste waters. Additionally, the effect of temperature, pH, redox conditions and the like may be considered. In all the cases, it is necessary to take into account the presence in the test water of other carbonate material. While it does not influence the absolute value of CO_2 assimilation, it can effect the values of radioactive uptake.

When studying the influence of toxicants on microorganisms, a nutrient medium is prepared for bacteria. Weak solutions of meat-peptone broth may be used. After sterilization and adjusting the pH value circum neutral conditions, the medium is inoculated with a young culture distributed into a series of 5-10 ml test tubes. The test is added to each at a required concentration, along with 0.1 or 1 ml of the labeled carbonate with an activity of $0.5\text{-}1.0 \times 10^6$ imp/min. The test tubes are then plugged with rubber stoppers and placed in an incubator for one day after which the samples are fixed with formalin and filtered through a membrane filter which retains the bacteria. After treatment with weak hydrochloric acid (1 percent solution) the filters are dried and the radioactivity is measured. The effect of various concentrations of the tested substance on bacteria is indicated by the radioactivity. The samples without the test substance serve as controls. Their radioactivity is assumed to be 100 percent. The results of one of such experiment on the action of silver ions are shown in Figure 4. Silver concentrations of 10^{-8} - 10^{-7} M have no effect on bacteria. The influence of these ions was detected beginning with the concentration 10^{-6} M, with complete inhibition of metabolism occurring at a concentration of 10^{-5} M.

This scheme may be used for determination of the toxicity of waste waters. When the presence thermostable substances is expected, the samples may be heated to 80 C for 10 min. to kill microflora, and then be added into test tubes with indicator organisms. Alternatively, one may eliminate preliminary heating and consider the control.

Determination of the Reserve and Rate of Consumption of Organic Substances According to the Method of Right and Hobbie

The living activity of microorganims may be estimated by the intensity of consumption of labeled organic compounds (Right and Hobbie, 1965). The method is based on the regularities of enzyme reactions. It allows the determination of the speed of circulation of separate organic substances and their reserves. Labeled glucose or acetate are most frequently used for this purpose.

The water to be tested is poured into 9 bottles of 30-50 ml each which are arranged in two parallel rows, four in each, with one remaining for control of the purity of the labeled substance. To the first two bottles are added 0.05 ml of the solution, to the second two bottles, twice the greater amount and so on. To the 9th bottle is added a fixative (Lugol's solution or formalin) and 0.1 ml of the solution of labeled substance.

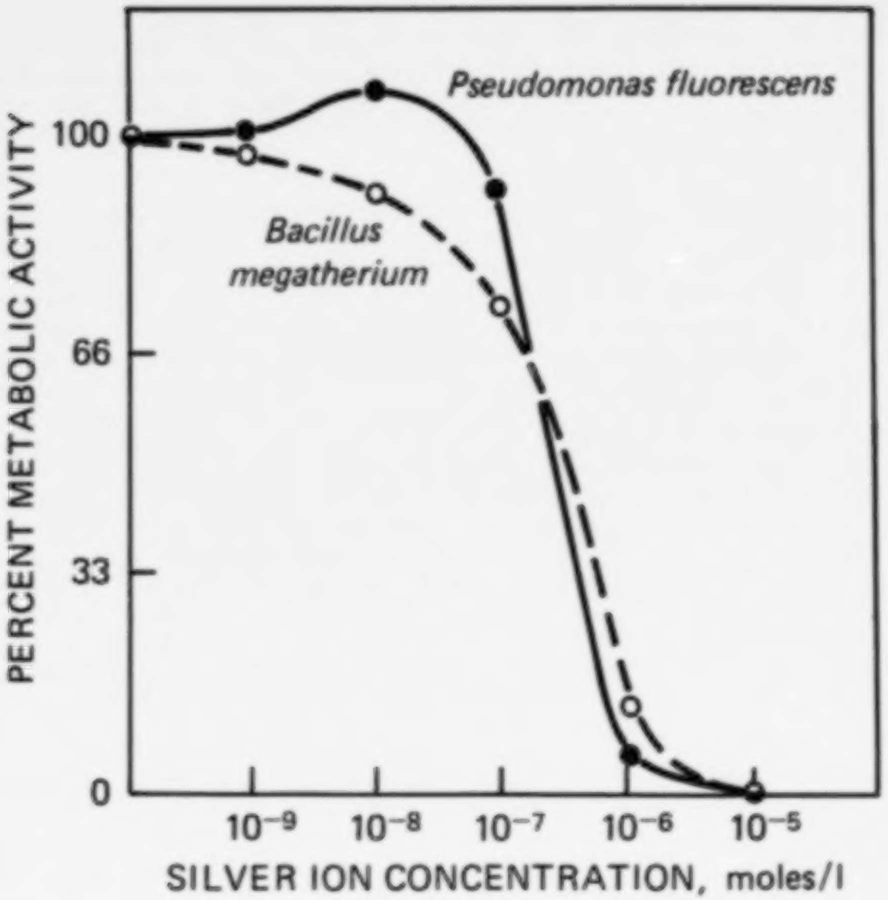

Figure 4. Effect of Ag ions on bacteria. Analysis using the heterotrophic assimilation of CO_2.

Incubation is accomplished for one hour at high temperature, or 3-5 hrs. at low temperatures. The samples are then fixed with Lugol's solution or formalin, filtered through membrane filters and 5-10 ml of physiological solution is passed through the filter. After drying, the radioactivity of microorganisms is determined and the data plotted. On the ordinate the values $\frac{R \cdot t}{r}$ are plotted, where R is the radioactivity of the added organic substance, t - time of incubation, r - radioactivity of organisms in the samples. The abscissa displays the concentration of the added organic substance. The points are connected with a line. The segment on the abscissa to the left zero on the ordinate axis (Figure 5) corresponds to the reserves of organic matter in the tested water.

The rate of consumption of a given substance may be calculated by the fomula:

$$V = \frac{r\,(A + S)}{Rt},$$

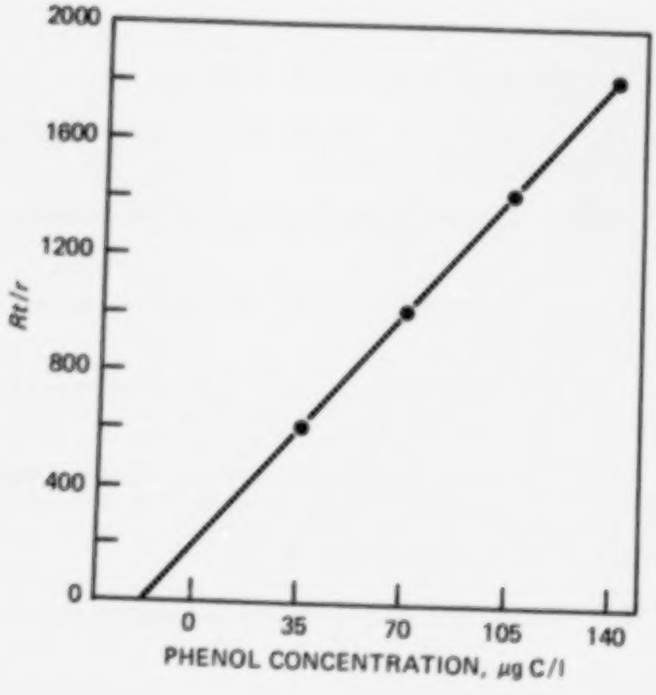

Figure 5. Determination of the reserves of phenol in the water of the Kanskoe Reservoir by the method of Right and Hobbie.

where:

V - rate of consumption of a substance (μg l/hr),
r - radioactivity of microorganisms in the whole sample,
(A+S) - quantity of organic matter in the sample:
A - added into the sample,
S - found on the graph (μg/l),
R - radioactivity of the added substance,
t - time of incubation.

This method may be used not only for the determination of the activity of microflora, but also for the reserves and the rate of consumption of separate toxic substances. For example, this technique was applied for determination of the content and the rate of consumption of phenol in water. Figure 5 shows the results of one experiment on the reserves of phenol in the water of the Kamskoe reservoir. The points fit a linear progression which cults off a segment of the abscissa equal to 13 mg/l C of phenol.

This method may also be used for the analysis of other organic toxicants. It is necessary only to have a corresponding ^{14}C-labeled subbstance.

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SECTION 7

AMMONIA AND NITRITE TOXICITY TO FISHES

Rosemarie C. Russo and Robert V. Thomann

AMMONIA

Introduction

Ammonia is a serious pollutant to aquatic life. It enters natural water systems from several sources, including agricultural and industrial wastes, and inadequately oxidized sewage effluents. Ammonia is also a natural biological degradation product of nitrogenous organic matter.

The toxicity to fishes of aqueous solutions of ammonia or ammonium salts is attributed to the un-ionized (undissociated) chemical species (NH_3) (Chipman, 1934; Wuhrmann, *et al.* 1947, Wuhrmann and Woker, 1948; Hemens, 1966), with the ionized species (NH_4^+) considered nontoxic, or significantly less toxic (Tabata, 1962). The concentration of un-ionized ammonia is dependent on the chemical and physical characteristics of the water, and therefore the toxicity of ammonia to fishes is dependent in part upon the effect of these variables on the aqueous ammonia equilibrium. The most important factors affecting this equilibrium are pH, temperature, and ionic strength. The concentration of un-ionized ammonia increases with increasing pH and temperature, and decreases with increasing ionic strength.

The toxicity of ammonia to fishes is also influenced by dissolved oxygen and free carbon dioxide. A decrease in the dissolved oxygen concentration increases the toxicity of ammonia (Downing and Merkens, 1955; Merkens and Downing, 1957), possibly because of increased ventilation by the fish and a corresponding increase in the rate of flow of ammonia across the gill tissues. Lloyd and Herbert (1960) reported that in waters of low CO_2 concentration the toxicity of ammonia may decrease, and attributed this to a reduction of pH at the gill membrane surface, brought about by the expiration of CO_2. Other factors which exert an effect on ammonia toxicity include previous acclimation of fish to low ammonia concentrations (Vamos, 1963; Malacea, 1968; Lloyd and Orr, 1969), physical stress (Herbert and Shurben, 1963), and fish size (Penaz, 1965). Several researchers have investigated the toxic effect of ammonia in combination with other poisons (Herbert, 1962; Herbert and Shurben, 1964; Herbert and Van Dyke, 1964; Vamos and Tasnadi, 1967; Brown, 1968; Brown, *et al.*, 1969). It is clear that the effects of the toxicants studies are generally additive; sometimes proportionally, but not always.

Aqueous Ammonia Equilibrium System

In aqueous ammonia solutions, un-ionized ammonia exists in equilibrium with the ammonium ion and the hydroxide ion. The equation expressing this equilibrium can be written as:

$$NH_{3(g)} + nH_2O_{(\ell)} \rightleftharpoons NH_3 \cdot nH_2O_{(aq)} \rightleftharpoons NH_4^+ + OH^- + (n-1)H_2O_{(\ell)}$$

As indicated in this equation, the dissolved ammonia molecule exists in hydrated form; it is hydrogen-bonded to at least three water molecules (Butler, 1964). The dissolved un-ionized ammonia is represented for convenience as NH_3; the ionized form is represented as NH_4^+; and total ammonia is the sum of these ($NH_3 + NH_4^+$).

The effect of pH and temperature on the aqueous ammonia equilibrium is significant. For example, a pH increase from 7.0 to 8.0 within the range 0-30 °C results in a nearly tenfold increase in the concentration of NH_3; a temperature increase of 5 degrees between 0-30 °C at pH 7.0 results in an NH_3 concentration increase of 40-50 percent.

There is no convenient method for measuring the concentration of NH_3 and NH_4^+ separately. However, if total ammonia concentration, pH, and temperature are known, the concentration of NH_3 may be calculated. Table 1 gives values of percent NH_3 in aqueous ammonia solutions of zero salinity.

TABLE 1. PERCENT UN-IONIZED AMMONIA IN AQUEOUS AMMONIA SOLUTIONS*

Temp.	pH Value								
(°C)	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	.0083	.0261	.0826	.261	.820	2.55	7.64	20.7	45.3
5	.0125	.0395	.125	.394	1.23	3.80	11.1	28.3	55.6
10	.0186	.0589	.186	.586	1.83	5.56	15.7	37.1	65.1
15	.0274	.0865	.273	.859	2.67	7.97	21.5	46.4	73.3
20	.0397	.125	.396	1.24	3.82	11.2	28.4	55.7	79.9
25	.0569	.180	.566	1.77	5.38	15.3	36.3	64.3	85.1
30	.0805	.254	.799	2.48	7.46	20.3	44.6	71.8	89.0

*[condensed from Thurston, *et al.* (1974)]

In natural waters with low to moderate amounts of dissolved solids (200-1000 mg/liter), this effect will slightly lower the concentration of NH_3. The magnitude of this effect will vary with the composition of the water in question. For a water of high pH (8-9) and total dissolved solids (TDS) of 500 mg/liter, which are predominantly calcium salts, the effect on the fraction of NH_3 present is approximately the same as if the temperature were lowered one degree. For waters of lower pH (5.5-6), but still high in calcium, somewhat higher values of TDS (600-700 mg/liter) would be required to produce a similar effect. For waters in which sodium chloride is the dominant ionic species, approximately twice these amounts of TDS would be necessary to produce a change comparable to a one-degree drop in temperature.

Toxicity of Ammonia to Fishes

Concentration values for ammonia toxicity tests on fishes have been variously reported as NH_3, NH_3-N, NH_4OH, total ammonia, total ammonia nitrogen, and formula weight for ammonium salts. Calculation of the percentage of total ammonia as un-ionized ammonia has also been made in a variety of ways, sometimes incorrectly. Recalculation of reported values is not always possible because of failure to report essential water chemistry parameters. Nonetheless, certain trends have developed which give some approximation of lethal levels of ammonia for salmonids and some species of warm water fishes.

In the case of short-term tests on rainbow trout (*Salmo gairdneri*) fry and fingerlings, median lethal concentration (LC_{50}) values as low as 0.2 mg/liter NH_3 have been reported (Liebmann, 1960; Danecker, 1964). Other researchers have reported LC_{50} values ranging between 0.3-0.6 mg/liter NH_3 for tests of one day or less on rainbow trout (Lloyd and Herbert, 1960; Herbert and Shurben, 1963, 1965; Ball, 1967; Lloyd and Orr, 1969; Smart, 1976), on brown trout (*Salmo trutta*) fry (Penaz, 1965), and on Atlantic salmon (*Salmo salar*) smolt (Herbert and Shurben, 1965).

In the case of short-term tests on fishes other than salmonids, Hazel, *et al.* (1971) reported 4-day LC_{50} values of 1.4 mg/liter NH_3 for striped bass (*Morone saxatilis*) and 1.0 for sticklebacks (*Gasterosteus aculeatus*). Colt (1974) reported 4-day LC_{50} values ranging from 2.4-3.8 mg/liter NH_3 for channel catfish (*Ictalurus punctatus*), and LC_{50} values ranging from 1.9-3.4 have been observed for fathead minnows (*Pimephales promelas*) (Thurston, unpublished data). A 17-hour LC_{50} of 1.3 mg/liter NH_3 was reported for Gambusia (*Gambusia affinis*) (Hemens, 1966), and a 24-hour LC_{50} of 2.9 was reported for channel catfish (Robinette, 1976). Lower LC_{50} values between 0.35-0.50 mg/liter NH_3 have been reported for 5- to 7-day tests on bream (*Abramis brama*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), and rudd (*Scardininus erythrophthalmus*) (Ball, 1967). In a longer test on rudd (Water Pollution Research, 1971) the LC_{50} value for 7 days was 0.5 mg/liter NH_3 and for 95 days was 0.24.

There are litle published data available on longer-term mortality tests for fishes of any species. A three-month test on 200 rainbow trout (Water Pollution Research, 1967) showed that 15 percent died at 0.22

mg/liter NH_3, and 5 percent died at both 0.11 and 0.06. In four separate tests of 3-5 weeks' duration, the LC_{50} values for rainbow trout fry were between 0.5 and 0.6 mg/liter NH_3 (Thurston, unpublished data).

Deleterious effects of ammonia at sublethal concentrations have been observed by a number of researchers. Reichenbach-Klinke (1967), in a series of one-week tests on 240 fishes of 9 species at concentrations of 0.1-0.4 mg/liter NH_3, observed swelling of and diminishing of the number of blood cells, inflammations, and hyperplasia, irreversible blood damage occurred in trout fry at 0.27 mg/liter NH_3. He also noted that these low NH_3 doses inhibited the growth of young trout and lessened their resistance to diseases. Smart (1976) observed a high incidence of disease, as well as gill damage, in rainbow trout exposed to 0.30-0.36 mg/liter NH_3 for up to 36 days. Flis (1968) reported that a 35-day exposure of carp (*Cyprinus carpio*) to a concentration of approximately 0.1 mg/liter NH_3 resulted in extensive necrobiotic and necrotic changes and tissue disintegration in various organs.

Reduction in growth rates for rudd has been observed after 95 days at concentrations greater than 0.1 mg/liter NH_3 (Water Pollution Research, 1971) and for channel catfish at 0.14 mg/liter NH_3 after 27 days (Robinette, 19/6). Smith and Piper (1975) reported a reduction in growth rates after 6 months and severe pathological changes in gills and livers of rainbow trout after 12 months' exposure at 0.2 mg/liter NH_3. For the 21-day period between egg hatching and swim-up stage, a reduction in development of rainbow trout (length, weight, and sac absorption) was observed at concentrations of 0.07 mg/liter NH_3 and higher (Thurston, unpublished data). Concentrations as low as 0.002 mg/liter NH_3 have been reported to cause gill hyperplasia in fingerling chinook salmon (*Oncorhynchus tshawytscha*) in 6 weeks (Burrows, 1964.

Rainbow trout have successfully spawned in the laboratory at 0.06 mg/liter NH_3 and have produced significant numbers of viable fry (Thurston, unpublished data).

NITRITE

Introduction

Nitrite is present in only trace amounts in most natural freshwater systems. In the process of nitrification, *i.e.*, the biological oxidation of ammonia to nitrate, nitrite is produced as an intermediate product. Primary treatment sewage plants discharge large quantities of ammonia and partially converted ammonia into receiving waters, and as the nitrification process proceeds downstream from the discharge point, nitrite levels above normal may be detected. Of the total nitrogen being discharged by a secondary treatment sewage plant, a lesser percent will be ammonia and a higher percent will be nitrate, but also the percentage of nitrite will increase. This percentage is related, in part, to how complete the nitrification process has been within the plant before discharge. In some cases, the amount of nitrite being discharge may raise the concentration of nitrite in a receiving water so that it may be significant to the stream biota. Water reuse systems in some fish hatcheries also employ the nitrification process to reduce ammonia concentrations. Where these systems are used, hatchery fish may also be subjected to increased nitrite levels. It has been shown (Smith and Russo, 1975) that nitrite induces methemoglobinemia in rainbow trout. This results in a reduction in the oxygen-carrying capacity of the blood, and fish may die from anoxia.

Toxicity of Nitrite to Fishes

The amount of published information on nitrite toxicity to fishes is small, and most available data are from static tests of short duration; many for 48 hours or less.

Of 13 fish species tested by McCoy (1972), logperch (*Percina caprodes*) was the most sensitive, dying in less than 3 hours at 5 mg/liter NO_2-N; the common white sucker (*Catostomus commersoni*) was the least sensitive, surviving 48 hours at 100 mg/liter. A 96-hour LC_{50} of 1.5 mg/liter NO_2-N was reported for mosquitofish (*Gambusia affinis*) (Wallen, *et al.*, 1957), and 10 mg/liter NO_2-N was reported to be fatal to minnows (*Phoxinus laevis*, *Agas*) in 14 days (Klingler, 1957). Channel catfish (*Ictalurus punctatus*) were studied by Konikoff (1975) and Colt (1974), who reported 96-hour LC_{50} values of 7.5 and 12 mg/liter NO_2-N, respectively.

The LC_{50} values for 96 hours for rainbow trout (*Salmo gairdneri*) ranged from 0.2 to 0.4 mg/liter NO_2-N, with an asymptotic LC_{50} of 0.14-0.15 (Russo *et al.*, 1974). The susceptibility of cutthroat trout (*Salmo clarki*) to nitrite appears to be comparable to that of rainbow trout. Observed 96-hour LC_{50} values for cutthroat trout were 0.5-0.7 mg/liter NO_2-N, with asymptotic LC_{50} valus of approximately 0.4 (Russo and Thurston, 1975). Chinook salmon (*Oncorhynchus tshawytscha*) exhibited 40 percent mortality in 24 hours at 0.50 mg/liter NO_2-N (Smith and Williams, 1974), and 96-hour LC_{50} values for chinook salmon were reported to be 0.88 mg/liter NO_2-N (Westin, 1974).

There are differences in the reported susceptibilities of fishes to nitrite. There does appear to be some genuine variability among fish species, and there may be differences depending on fish size. It should be pointed out that in some cases differences may be due to variations in test water conditions. Recent work (Russo and Thurston, unpublished data) has shown a wide variation in lethal concentrations of nitrite in waters of different pH and salinity.

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SECIION 8

A RESEARCH SYSTEM FOR DEVELOPING FISHERIES STANDARDS FOR WATER QUALITY, CONSIDERING THE PECULIARITIES OF TRANSFERRING EXPERIMENTAL DATA TO NATURAL WATER BODIES

L.A. Lesnikov

At present in the USSR, two groups of standards of water quality are being developed with the purpose of protection of the waters from pollution: 1) sanitary hygienic, and 2) fisheries MPC (maximum permissible concentrations). Both groups of the MPC are to be approved by the Ministries of Health of the USSR, and Fisheries of the USSR respectively, and will be official interdepartmental standards.

When setting fisheries standards, complex investigations are performed on water-bodies, and in laboratories. The latter are most used, since only experimental work enables the investigator to establish clearly the relation between concentrations of pollutants, and the degree of the disturbance in organisms.

The results of field investigations may be taken into account, but only for comparison, since alterations in organism response are always the result of the actions of not only the pollutants, but also of other natural and anthropogenic factors. In addition, under experimental conditions, it is possible to evaluate the effect of substances on the living functions of organisms, while in natural waters the influence of pollution is not direct, but functions through environmental and ecological conditions.

In the USSR there are more than 100 fisheries standards. In 60 percent of cases the fisheries standards are close to the corresponding sanitary-hygienic ones, but in 40 percent they are more stringent, occasionally 10-100 times more restrictive (Lesnikov, 1974).

In the USA, only recently investigations were started on the chronic action of low concentrations of pollutants on aquatic organisms (Brungs, 1972). In the USSR, such investigations have been performed since 1938-1939 (Eltsina, 1939; Stroganov, 1940; Stroganov, 1941).

From the very beginning, elaboration of the fisheries MPC in the USSR was based on the following principles formulated by N.S. Stroganov (1941):

1. To investigate, as far as possible, the influence of tested substances on the whole life-cycle of aquatic organisms, or on its most susceptible stages.

2. To conduct observations for the entire period of a complete biological cycle (for crustaceans not less than 15 days), or of its separate stages.

3. To direct attention to the influence of toxic substances not only on survival, but also on the main physiological functions of an organism (heart beat, reproduction, breathing).

4. To use in the experiment various organisms, since different organisms respond to the action of substances in different ways.

5. To fix permissible levels in waters according to the weakest biological link.

Later, all the indices of the action of substances on organisms were subdivided into two categories, principal and supplemental. The principal category describes those characteristics of the existance of populations of organisms under natural conditions which are well understood, i.e., rate of growth, reproduction, death. Supplementary indices are used to clarify the character of action of a given substance. The supplementary indices are very important in order to establish the causes of death of organisms in natural waters.

For deeper understanding of the character of action of a substance, it is necessary to perform experiments on more than one species of aquatic organism. When choosing test-organisms their role in the circulation of substances and their relative sensitivity to pollutants was considered. The organisms are divided into 3-4 groups according to their relative sensitivity to toxic substances (Lesnikov, 1968, 1969, 1975; Stroganov, 1971). Organisms primarily from the first two groups (the most sensitive organisms) are taken for the experiments.

In the USSR, a set of tests is used to study the influence of hydrochemical indices of water (gaseous and ionic composition of water, pH, content of organic substances, dynamics of nitrogen compounds, time of decomposition of pollutant) on:

1. Producers: *Scenedesmus quadricauda.*

2. Consumers: Zooplankton - *Daphnia magna* and *D. longispina.*
   Zoobenthos - *Chironomus dorsalis, Gammarus pulex.*
   Fish - *Salmo irideus, Coregonus peled* and others. (parallel experiments of influence on eggs, larvae, less than 1 year age class and in some instances, on yearlings).

Reducing bacteria -	number of bacteria and saprophytes growing on MPA (Lesnikov, 1973, 1975).

N.S. Stronganov (1975) added a series of experiments on aquatic plants (elodea and others) to these tests.

Investigations for each level are conducted to reveal the acute lethal, the chronic lethal and the sublethal (inhibitory) effect of toxicants. Analysis of the dependence of survival time of organisms on the concentration of toxic substances (Jones, 1957, 1964; Lesnikov, 1973, 1976) enables an estimation of the duration of acute (short-term) and chronic (long-term) experiments:

Type of Experiments	Invertebrates	Fish
Acute	10 days	15 days
Intermediate	1 month	3 months
Chronic	3 months	6 months or longer

The boundary between acute and chronic lethal concentrations was taken to be a characteristic bend of the curve. Between chronic lethal and sublethal concentrations, the position of the asymptote of Stroganov was used as the determining factor. The bend for different species and substances was typically between the 4-14th day of the experiment. The duration of chronic experiments was determined by the detected cases of the remote negative effect.

For some organisms (*Daphnia*, and certain algae) the methods of experimentation on populations are established. For *Daphnia*, the whole set of population reactions such as logrythmic growth of the biomass, a regular transition from parthenogenetic to bisexual reproduction, etc. are reproduced. It has been established that when a population reaches its saturation biomass, its sensitivity to pollutants becomes 3-3.5 times greater than before (Table 1).

TABLE 1. CHANGES IN BOUNDARY CONCENTRATIONS OF 3 POLLUTANTS INFLUENCING BIOMASS IN *DAPHNIA MAGNA*.
(Boundary concentrations are estimated on the basis of regression equation).

	Days of Experiment					
Pollutants	6-9	9-12	12-15	15-18	18-21	21-30
Sewage from oil refinery, %	5.0	1.7	1.4	0.7	1.6	-
Sewage from chemical plant, %	2.9	1.6	1.1	1.9	-	-
Cobalt chloride, mg Co^{++}/l	-	0.1	0.1	0.1	0.09	0.02

At present, the influence of more than 30 different substances on *Daphnia* populations have been investigated, in only two cases were exceptions observed. These exceptions were noted: 1) when studying the influence of sodium chloride on populations of *D. magna* (adaptation limits of the *Daphnia* to salination exceeded the increase in sensitivity), and 2) in the case of the influence of sulphate sewage from paper mills on *D. magna* (adaptation to organic substances). In the both instances, the experiments with *D. longispina* yielded typical results. This latter species adapts to a lesser degree to increased salinity, and is more sensitive to saprobic pollution. In the case of an increase of sensitivity of population to pollutants, it is necessary to deal with the increase of sensitivity of individuals in the moment when the saturation biomass in reached (Lesnikov, 1970). Four main types of effects of substances on the productive properties of population can be discerned:

Type 1. The substance increases mortality of individuals without disturbing the functions of growth and reproduction in individuals. This type is analoguous to the effect of predation and fishing on the population. To some extent, the death of some of the individuals is compensated by intensification of growth and reproduction in others. Thus, even the death of a part of the community may not lead to a notable decrease in the rate of growth of the biomass.

Type 2. The substance influences the rate of metabolism and, thus the rate of growth in weight of individuals, but the reproductive function remains undisturbed. Usually, the biomass of the test population is close to that of the control, but is attained at a later time.

Type 3. Reproductive function of the population is disturbed. The maximum biomass in the test population usually does not reach that of the control. In *Daphnia*, no cases of formation of ephippia during the period of high biomass are observed, although these processes take place in the control. This is the most dangerous type of the effect.

Type 4. Under the influence of substances, the biomass is higher than in the control. For the *Daphnia* populations, this is usually the influence of sewage which passed through biological treatment (discharge of a part of activated sludge increases the nutrient base for *Daphnia*). However, if the sewage is derived from industrial enterprises, the stimulation may be accompanied by evident intoxication of a part of the individuals. In addition, with this type of effect, it is necessary to account for the possibility of a change in the water quality which may render it unsuitable for valuable fish, e.g., the whitefishes and salmonids, which can be displaced by less valuable fish.

Evaluation of the influence of pollutants upon whole populations of aquatic organisms makes it easier to extrapolate the experimental data to natural waters. One may expect that similar disturbances will take place in nature.

It is necessary to take into account the fact that in nature, a pollutant acts to influence a number of other factors. The first attempt at classification of these factors was made by Wuhrman and Woker (1955). An improvement of this classification is offered in Table 2.

In addition, usually not one, but a number of pollutants are present in natural waters. It is generally accepted in the USSR that it is possible to estimate the sum of the effects of substances as an additive function. The cases of synergism and antagonism are to be accounted for only in acute lethal concentrations, since they are less important under the conditions of chronic action.

Pollutants exert simultaneous action on a number of species of organisms. The net result depends on the relative sensitivity of these species to the pollutant. Therefore, creation of a scheme of classification of organisms according to their sensitivity to toxicants is directly related to the question under consideration. The relation of organims to saprobic pollution is well considered in various saprobity systems. The relation to toxicants requires special elaboration. Here a scheme of division is proposed (Table 3) accounting for analogous elaborations in the USSR (Stroganov, 1971) and in the USA (Muirhead-Thomson, 1971).

A more detailed division of organisms according to their sensitivity is desirable, but difficult, for two reasons: 1) the indicated classification is a generalization, and the specific position of many organisms requires further clarification, since within each of the groups there are further gradations of sensitivity; and 2) the sensitivity of single species to various types of toxic substances is different. It is possible that future considerations will require the creation of not one, but a number of such systems, while more useful for classification, such an addition will make the system more cumbersome.

Thus, in analyzing the actual differences between the conditions in experiments and those of natural waters, the experimental data can be used with greater assurance if the analytical situation employs natural conditions.

TABLE 2. THE INFLUENCE OF FACTORS ON THE CHARACTER OF ACTION OF THE POLLUTANTS ON AQUATIC ORGANISMS

Type of Influence	Factors	Character of Influence
On properties of pollutants.	CO_2 content in water.	Rate of oxidation of pollutants.
	Content of mineral substances in water.	Coagulation of some substances, neutralisation of acids and alkalis.
	Content of organic matter.	Formation of complex compounds with pollutants absorption.
	pH of the water.	Change in the degree of dissociation of pollutants, and in their toxicity.
	CO_2 content.	Change in the direction of chemical reactions and buffer properties of water.
On the time and condition of the contact or organisms with pollutants.	Dynamics of water masses (velocity of currents, convection, stagnation, etc.).	Dilution of pollutants, possibility of their concentrating in certain parts in water or in bottom sediments.
	Distribution and migrations of organisms in a water-body.	Possibility of organisms migrating into polluted water.
On sensitivity of organisms to the action of toxicants.	Size, age, sex.	Differences in sensitivity of stages. Usually males are more sensitive than females.
	Contents of other substances in water.	Antagonism and synergism of toxic substances.
	Discrepancy between requirements of organism and environmental conditions.	Increasing sensitivity to injuring factors, including the action of pollutants.
	Weakening of organisms due to starvation, parasites, infections, etc.	The same as above.
	Stress conditions of organism.	Changes in time of reaction and in intensity of injuring.

TABLE 3. DISTRIBUTION OF ORGANISMS ACCORDING TO THEIR RELATIVE SENSITIVITY TO TOXIC SUBSTANCES (TOXOBITY)

Group of Organisms		Toxobity			
Ecological	Taxonomic Group	Oligotoxobity	Betamesotoxobity	Alphamesotoxobity	Polytoxobity
Fishes	Salmon	All species			
	Whitefish	All species			
	Perch	Zander	Caspian zander, perch, ruffe		
	Sturgeon	All species			
	Carp		Bream, white bream, roach bleek	Carp, crucian carp, tench bighead, amur loach	
	Sheatfish		Sheatfish		
	Pike		Pike	Pike	
	Eel				
	Cod		Burbot		
	Stickleback		Stickelback		
Zooplankton	Watermites			All species	
	Cladocerans		Daphnias, Sidas, predatory cladocerans	Chidorids Bosminids	
	Ostracods		All species	All species	
	Copepods		Calanoids	Cyclopoids	
	Rotifers		All species	Bdellids	
	Ciliates			Mobile forms	Mobile forms
	Zooflagellats			All species	All species
Zoobenthos	Crustaceans	Gammarids mysids, corophiids, crayfish			
	Harpacticides		All species	All species	
	Mollusks		Bivalvia	Gastropoda	
	Aquatic Insects	Ephemeropterans	Mayflies, Draggonflies, Caddis Flies	Chironomids, Beetles, Bugs Culicines	
	Aquatic Worms			Oligochaets Leeches, Planaria	Tubificids Lumbriculids

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SECTION 9

COLLAGEN AND HYDROXYPROLINE IN TOXICOLOGICAL STUDIES WITH FISHES

Foster L. Mayer and Paul M. Mehrle

Chronic toxicity studies with fish are expensive, high-risk endeavors, requiring from 10 months to one year to conduct. Such studies include growth, reproduction, and survival of adults, and growth and survival of the offspring. As a consequence, there has been much interest in the development of alternative methodologies that provide similar information with less expenditure of time and effort. Grant and Schoettger (1972) stated that the monitoring in fish of biochemical factors that can be correlated with toxicant exposures and residues, provides a useful means of anticipating the subtle, adverse impacts of organic contaminants on the fish. To date, however, investigators have used biochemical measurements alone in many studies to arrive at rather broad conclusions, without determining the ultimate effects on growth, reproduction, and survival (i.e., whether the chemical changes observed were within the adaptive capacity of the fish). Therefore, attempts were made to assess the possibility of using biochemical factors as indicators or predictors of growth and development in fish, thus decreasing the time required for chronic toxicity determinations. Growth of fish is usually evaluated by measuring weight and length; however, biochemical changes due to intoxication should occur before reductions in growth. As potential indicators of growth and development, backbone collagen and the hydroxyproline concentration in collagen were selected, and these measurements were incorporated into basic studies with toxaphene to provide information for the establishment of water quality criteria (Mayer *et al.*, 1975, 1977; Mehrle and Mayer, 1975a, 1975b, 1976).

RATIONALE FOR MONITORING HYDROXYPROLINE AND BACKBONE COLLAGEN

Collagen is the major fibrous protein of all vertebrates and most of the invertebrate phyla (Piez and Likens, 1958). Its most important function in vertebrates is to serve as the major component of the organic matrix of connective tissues and bones. The collagen molecule is unique in its amino acid content (Harrington and Hippel, 1963); the amino acids hydroxyproline and proline combined make up about one-tenth and glycine another third of the total amino acid composition in collagen. In animal tissues, hydroxyproline is found only in the protein's collagen and elastin. Since the total amount of elastin is very small in comparison with that of collagen, and since the hydroxyproline content of elastin is only about one-tenth that of collagen, its contribution to the total hydroxyprolin content is negligible (Green *et al.*, 1968). The synthesis of collagen, like that of other proteins, occurs on the ribosomes in fibroblasts, osteoblasts, and chondroblasts. However, hydroxyproline and hydroxylysine in the collagen molecule are not derived from incorporation of the free amino acid into the polypeptide, but instead are derived from the hydroxylation of their respective precursors, proline and lysine, after incorporation of proline and lysine into the polypeptide protocollagen. The enzyme collagen hydroxylase, or peptidyl proline hydroxylase, which begins its activity during gastrulation, catalyzes hydroxylation; vitamin C, α-ketoglutarate, and ferrous ion serve as cofactors for the enzyme (Mussini *et al.*, 1967).

The importance of collagen in animals in shown by its wide distribution and many functions during growth and development. One of its major functions is to serve as the structural support for bones. Dried bone consists of one-third organic matrix and two-thirds minerals. About 90 percent of the organic matrix is collagen, and the rest consists of mucopolysaccharides, mucoproteins, and lipids (Nusagens *et al.*, 1972). Calcification and mineralization take place around and within the collagen fibrils in bone and, as development proceeds, the deposition of calcium and phosphate produces mature bone.

The use of collagen as a representative "differentiated" protein in the study of embryonic development has been reported in amphibian embryological investigations (Green *et al.*, 1968; Rollins and Flickner 1972). Collagen synthesis, though repressed during the first cleavage stages of the embryo of a frog (*Xenopus laevis*), begins during gastrulation and increases 500-fold through neutrulation, hatching, and posthatching stages. Also, decreased hydroxyproline excretion in urine has shown promise as a detector of nutritional deficiency and reduced growth rates in humans (Whitehead and Coward, 1969). Fish continue to grow throughout life, and their vertebrae continue to elongate and enlarge with growth. It has therefore hypothesized that backbone development should increase in proportion to increase in growth, and that increases in collagen and hydroxyproline would be indicators of this growth.

RELATION OF COLLAGEN AND HYDROXYPROLINE TO GROWTH

Brook trout (*Salvelinus fontinalis*), fathead minnows (*Pimephales promelas*), and channel catfish (*Ictalurus punctatus*) were continuously exposed to toxaphene in water; the proportional diluter systems used were modeled after Mount and Brungs (1967) and modified as recommended by McAllister *et al.* (1972). The diluter systems delivered five concentrations of toxaphene, with a dilution factor of 0.5 between the concentrations, and a control (Table 1). We used flow-splitting chambers as designed by Benoit and Puglisi (1973) to thoroughly mix and divide each toxaphene concentration for delivery to duplicate exposure tanks. Artificial daylight was provided by the method of Drummond and Dawson (1970), and water temperatures were maintained within ± 0.2 °C.

TABLE 1. SUMMARY OF EXPERIMENTAL CONDITIONS AND SAMPLING PERIODS USED DURING CONTINUOUS EXPOSURE OF BROOK TROUT, FATHEAD MINNOWS, AND CHANNEL CATFISH TO TOXAPHENE[a].

Species and Life Stage	Toxaphene Concentration (ng/l)	Water Temperature (°C)	Age At Initiation of Exposure (Days)	Duration of Exposure (Days)	Growth Determinations (Days)	Biochemical Determinations (Days)
Brook trout						
Eggs	39-502	9	eyed eggs	22 before hatch		
Fry				90	30, 60, 90	7, 15, 30, 60, 90
Fathead minnows						
Experiment 1	94-727	25	10	150	60, 90, 150	150
Experiment 2	13-1/3	25	40	295	30, 98	98
Eggs			0[b]	5		
Fry			0	30	30	30
Channel catfish						
Adults	49-630	12-26	2.5 years	100	50, 100	100
Eggs		26	0[b]	7		1
Fry		26	0	90	5, 30, 60, 90	15, 90

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[a] Experimental details are given by Mayer *et al.* (1975, 1977) and Mehrle and Mayer (1975a, and 1975b).

[b] Eggs and fry were produced and remained in the exposure units.

Hydroxyproline and backbone collagen were found to be sensitive biochemical indicators of growth in fathead minnows exposed to toxaphen (Table 2). The correlation between hydroxyproline in backbone collagen and fish weight was high, with a coefficient of determination (r^2) of 0.982, and the relation between collagen concentration and weight was even higher (r^2, 0.990). Coefficients of determination were also relatively high for brook trout and channel catfish. The measurement of hydroxyproline has some advantages over the measurement of collagen in that hydroxyproline can be directly determined in eggs and whole fry, whereas collagen is determined indirectly, except in fish large enough to permit analysis of the backbone itself. The impact of toxicants on collagen and hydroxyproline metabolism is probably greatest during early life stages of fish because the young fish are generally more sensitive than older fish to toxicants, and have more rapid developmental rates. However, in a preliminary study with the dimethylamine salt of 2,4-D and fathead minnows (Mayer and Mehrle, 1974), it was found that hydroxyproline changed little in backbone collagen, but that collagen itself decreased significantly ($P<0.05$). These results indicate that, when possible, both hydroxyproline and collagen in backbone should be measured to facilitate toxicological interpretation.

The use of collagen and hydroxyproline as predictors of growth effects shows some promise, but has not been fully delineated (Table 3). The reduction of growth caused by toxaphene in brook trout occurred 23 to 30 days after effects were observed in hydroxyproline content. In the first study (Mehrle and Mayer, 1975a), the growth, and the collagen and hydroxyproline concentrations in adult fathead minnows were significantly reduced ($P<0.05$) at all toxaphene concentrations. The hydroxyproline concentration in backbone collagen of adults was significantly reduced ($P<0.05$) in toxaphene concentrations as low as 54 ng/l, whereas growth was significantly reduced only in the 97 and 173 ng/l exposures of a second study (Mayer *et al.*, 1977). In the resulting fry, however, growth was more sensitive than hydroxyproline as an indicator of the effects of toxaphene. Growth of channel catfish fry was not reduced by toxaphene until 30 days after the eggs hatched, but the hydroxyproline content of eggs from exposed adults was significantly reduced ($P<0.05$). The effects of toxaphene on hydroxyproline first appeared at exposure concentrations of 72 ng/l, whereas effects on growth first appeared at 299 ng/l. However, the reduction in hydroxyproline was related to bone development, and numerous fish in the 72 through the 630 ng/l exposures had broken backs (Mayer *et al.*, 1977; Mehrle and Mayer, 1976). Also, survival was significantly reduced ($P<0.05$) in concentrations of 792 to 630 ng/l and

TABLE 2. RELATION BETWEEN BACKBONE DEVELOPMENT AND WEIGHT IN FISH EXPOSED TO TOXAPHENE

Species	Coefficient of Determination (r^2) Hydroxyproline	Coefficient of Determination (r^2) Collagen	P Value of Correlation Coefficient (r)
Brook trout	0.962	0.964	0.005
Fathead minnow	0.982	0.990	0.0005
Channel catfish	0.974	0.982	0.0005

TABLE 3. STATISTICAL SIGNIFICANCE[a] OF THE EFFECTS OF TOXAPHENE ON GROWTH AND HYDROXYPROLINE CONCENTRATION IN FISH.

Species, Life Stages and Exposure Period (Days)	Exposure Concentration (ng/l) of Toxaphene, and Statistical Significance (1) or Nonsignificance (0) of Effects on Growth (Left Column) and Hydroxyproline Concentration (Right)				
Brook Trout	39	68	139	288	502
Fry					
0	0 -[b]	0 -	0 -	0 -	0 -
7	- 0	- 1	- 1	- 1	- 1
15	- 0	- 1	- 1	- 1	- 1
30	0 1	0 1	1 1	1 1	c
60	1 1	1 1	1 1	c	c
90	1 1	1 1	1 1	c	c
Fathead Minnows	13	25	54	97	173
Adults					
98	0 0	0 0	0 1	1 1	1 1
Fry					
30	0 0	0 0	1 0	1 1	1 1
Channel Catfish	49	72	129	299	630
Adults					
100	0 0	0 0	0 0	0 0	0 0
Eggs					
7	- 0	- 1	- 1	- 1	- 1
Fry					
5	0 -	0 -	0 -	0 -	0 -
15	0 0	0 1	0 1	0 1	0 1
30	0 -	0 -	0 -	1 -	1 -
90	0 0	0 1	0 1	1 1	1 1

[a] Statistical significance, P=0.05.

[b] Not determined.

[c] All fish had died.

growth rates of the surviving catfish fry may not have been fully representative of the original populations.

EFFECT OF ORGANOCHLORINE CONTAMINANTS ON COLLAGEN FORMATION

Various reports have stated that vitamin C is involved in the hydroxylation of drugs and chemicals in the liver of mammals (Axelrod *et al.*, 1954; Levin *et al.*, 1960; Street *et al.*, 1971; Wagstaff and Street, 1971), and in collagen formation by the hydroxylation of proline to hydroxyproline (Barnes, 1969; Barnes *et al.*, 1970; Mussini *et al.*,; Peterkofsky, 1972) (Figure 1). Since these two hydroxylation processes may compete for the available vitamin C, an attempt to determine the effects of toxaphene on the distribution of vitamin C in liver and bone in channel catfish was made (Mayer and Mehrle, 1977). Ten-month old fish was continuously exposed to 37 to 475 ng/l of toxaphene, and to exaggerate the response, a diet low in vitamin C was fed to all test fish. The diet contained 63 mg of vitamin C per kilogram of food, rather than the recommended amount of 100 mg/Kg (National Academy of Sciences, 1973). Vitamin C in liver and bone, and collagen in bone were determined after 90 and 150 days of exposure.

Within each time period, the vitamin C content of the liver remained rather constant, regardless of the toxaphene exposure (Table 4). Vitamin

TABLE 4. MEAN CONCENTRATIONS OF VITAMIN C (μg/g OF WET TISSUE) IN LIVER AND BACKBONE AND COLLAGEN (mg/g OF DRIED BONE) IN THE BACKBONE OF CHANNEL CATFISH FED A DIET LOW IN VITAMIN C, AFTER 90- AND 150-DAY EXPOSURES TO DIFFERENT CONCENTRATIONS OF TOXAPHENE.

Toxaphene Concentration ng/l	Vitamin C Concentration					
	Liver		Bone		Collagen	
	90 Days	150 Days	90 Days	150 Days	90 Days	150 Days
0 (control)	39 (7)[a]	51 (5)	23 (7)	4.9 (0.6)	320 (18)	370 (11)
37	28 (3)	46 (3)	8.2* (2.9)	6.5 (1.3)	400 (21)	290* (13)
68	47 (4)	49 (7)	12* (2)	4.5 (1.9)	290 (10)	330* (16)
106	33 (8)	65 (7)	7.8* (2.7)	8.0 (0.7)	290 (14)	310* (19)
218	46 (3)	57 (10)	13* (4)	4.2 (0.9)	310 (25)	340* (25)
475	34 (3)	52 (11)	5.0* (2.6)	6.0 (1.0)	280 (20)	280* (6)

[a] Standard error.

*Significantly different from controls ($P<0.05$).

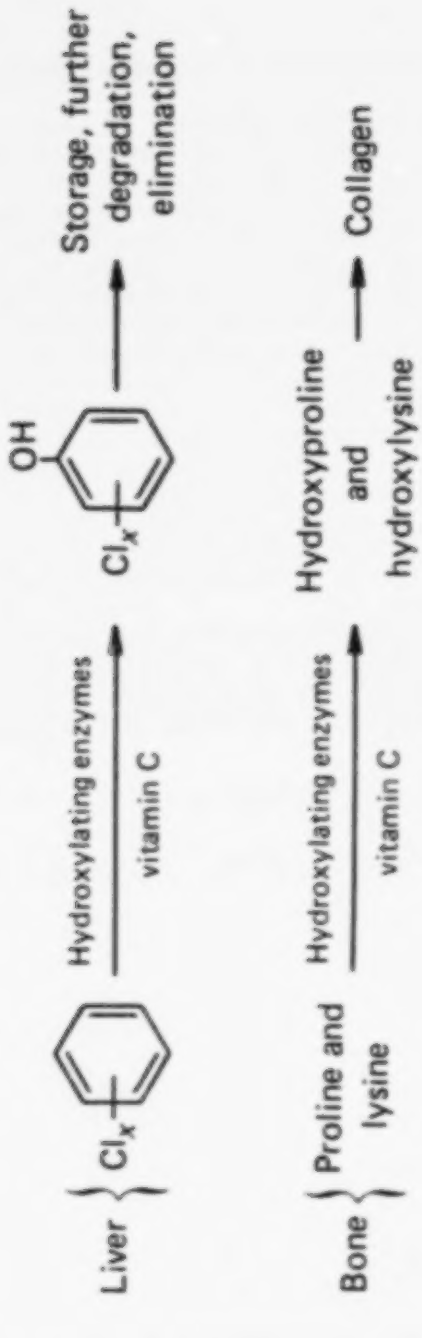
Liver
Cl$_x$
Hydroxylating enzymes
vitamin C
OH
Cl$_x$
Storage, further degradation, elimination
Bone
Proline and lysine
Hydroxylating enzymes
vitamin C
Hydroxyproline and hydroxylysine
Collagen

Figure 1. Hydroxylative enzyme systems in liver and bone that require vitamin C.

C in bone, however, was significantly reduced ($P<0.05$) in fish exposed to toxaphene for 90 days, and was low in all fish, including the controls, at 150 days. This response in the controls was probably due to the chronic effects of the diet itself. After 90 days exposure, backbone collagen was significantly reduced ($P<0.05$) only in the highest concentration; at 150 days, however, it was significantly reduced in all toxaphene treated fish. Thus, when fish are exposed to an organochlorine contaminant such as toxaphene, the increased use of vitamin C by the liver in hydroxylative detoxication mechanisms may reduce the amount in the bones by as much as 50 percent. This reduction of vitamin C in bone is believed to inhibit the formation of hydroxyproline from proline and reduces collagen formation.

CONCLUSIONS

Biochemical characteristics such as hydroxyproline and collagen concentrations in bone can be used as indicators, and within limits, predictors of growth in fish thereby shortening chronic toxicity tests. Although growth can be directly related to collagen and hydroxyproline metabolism in fish, the mechanism by which growth is reduced is not known. Other biochemical processes requiring vitamin C could also be affected when large amounts of the vitamin are used by the liver in detoxication of organic contaminants through microsomal hydroxylative enzymes.

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SECTION 10

EXPERIMENTAL TESTING OF TOXICITY OF WATER MEDIA AND INCREASING OF THE SENSITIVITY OF BIOLOGICAL TESTS

L.P. Braginski, V.D. Bersa, T.I. Birger, I.L. Burtnaya,
F.Ya. Komarovski, A.Ya. Malyarevskaya, E.P. Shcherban

General increase of anthropogenic pollution of the hydrosphere raises the problem of quantitative and qualitative characterization of pollutants and evaluation of their biological danger. Additionally, the question of establishing analytical and control methodologies of wide applicability for assaying the toxicity of the water medium based on evaluation of biological effects of toxicants is of paramount importance. One of the principal ways of solving the problem is the application of biologic tests. These tests have enjoyed wide spread acceptance in Europe (Bringmann and Kuhn, 1959; Liebmann, 1960; Stanislawski, 1969), in the USA (Katz, 1971; Environmental Protection Agency, 1972; Federal Water Pollution Control Administration, 1969) and in the USSR (Braginski, 1971; Lesnikov, 1971; Anon., 1959; 1971, 1966; Stroganov, 1971).

The main advantage of the biological tests are simplicity and availability of methodology, high sensitivity of the test organisms to the minimum concentrations of toxic agents, speed, and the fact that expensive reagents and equipment are not required. The main principle of biological testing is extremely simple. It is used to establish confident differences between the experiment (medium containing toxicant) and the control (clean water) in any indicative biological parameter test organism. Both alternative (life-death) and graded (percent of the experiment as contracted with the control) experiments are used to indicate complete or partial inhibition of essential functions of test organisms under the influence of the test water or toxicants in certain concentrations.

Discrimination between two types of test organisms is made: 1) indicative, and 2) representative ones. The first category implies the use of organisms with the greatest degree of sensitivity to toxicants, the second implies the use of organisms that most fully represent a given ecosystem (the crustacean *Epishura* in Lake Baikal, *Mysis* in the Danube estuaries, and so on).

When choosing test organisms, one is compelled to account for their individual ecological specificity, their restriction to certain type of waters, lentic or lotic environmental requirements, typical habitats (water, mud, vegetation thickets, etc.), and degree of sensitivity to toxicants. The latter is specific for each species and varies within a very great range: from nannograms to milligrams and grams per litre (Alekseev, 1970). Investigations of both Soviet (Alekseev and Antipin, 1976; Filimonova, 1974; Anon., 1975) and the filter feeders (especially the cladocerans) are the most sensitive test organisms, and hence, the most widely used. Different species of Cladocera, however, have their own specific sensitivity, and between the laboratory test cultures (pure lines) and natural populations there are also substantially great differences in resistence to toxicants.

In the world literature there are a number of biological tests suggested. These have been described in appropriate reviews (Katz, 1971; Stanislawska, 1969). Many of these tests have not received international recognition, and are being used primarily within national laboratories, or have been encorporated within the limits of regional agreements (Anon., 1966).

The present communication deals mainly with the authors' efforts, representing a contribution of one laboratory to the given problem. Since algae are used only rarely for toxicological testing, the topic will be discussed in some detail.

Test cultures of algae are grown either as pure cultures, or as samples from natural waters at the time of mass development of some species (e.g., *Stephanodiscus hantzchii* in spring, or *Microcystis aeruginosa* in summer). There may be essential differences between the results obtained on these species, since laboratory cultures are more delicate, and have been developed under artificial conditions, frequently on complex media. However, the benefits of uniform results and synchrony cannot be overlooked.

The simpliest test is the one involving the death of the unicellular algae in the presence of toxicants. A quantitative ratio of living to dead cells in the test culture is established by means of microscopy. Illustrative of this technique is the testing of various ions. For example, a solution containing copper and ammonium is so toxic that even at a concentration of 0.05 mg/l active from the living cells is practically absent, and at 0.5 mg/l no cells survived (Table 1).

TABLE 1. RESULTS OF THE TOXICITY OF A COPPER-AMMONIUM SOLUTION ON TEST CULTURE OF *MICROCYSTIS AERUGINOSA* (LABORATORY STRAIN, 5-DAY EXPERIMENT)

Concentration mg/l	Percentage		
	Living	Dead	Dying
Control	68	32	0.0
0.05	0	10.7	89.3
0.1	0	96.8	3.2
0.5	0	100	0
1.0	0	100	0

The method of low level luminiscence developed by a group of Moscow biophysicists enables an evaluation of the toxicity of the minimum concentrations of toxic agents.

In addition to the luminiscence method, differentiation of living and dead algal cells may be performed with a help of dyes (0.1 percent neutral red), reagents (TTX) and fluorochromes.

Occasionally, dead and living algae may be discerned even when using vital microscopy, or the dark field technique. These techniques reveal tological disturbances (plasmolysis, disintegration of the chromatophore and cell walls) under the action of toxicants in large filamentous algae *Cladophora*, *Rhizoclonium* and others (Braginski, 1972; Anon., 1959). The Institute of Biology of the Ukranian Academy of Sciences has proposed a number of cytochemical tests which enable observation of disturbances of the living activity of blue-green algae in a toxic medium. These methods involve determination of permeability of cell membranes when staining with nitrosine, determination of ascorbic acid content, sulphur hydryl group determination, measurement of enzymatic activity of cells, and observation of the redox potential of test cultures (Osterov, 1968).

Advances in understanding of the physiology of algae allow the use of a whole complex of experimental methods including the celloscopic counting of cells in test cultures, the determination of the chlorophylls, carotenoids and other pigments (Anon., 1975). All these tests are important since they take into account the possible harmful effect of toxicants, not only upon animals, but also upon the components of primary production of waters. To date, insufficient attention has been given to the primary producers when ascertaining the ecological threat of chemical pollutants. In this regard, of substantial interest are the investigations for direct determination of inhibition of photosynthesis (Knepp, 1969).

The authors proposed a number of modifications of the Knepp test, including the use of *Scenedesmus acuminatus*, *S. bjugatus*, and the diatom *Stephanodiscus hantzchii* (Bereza, 1972, 1973) instead of *S. quadricaudus* as test species, and a modification of the oxygen method for determination of primary production and destruction of phytoplankton in the presence of toxicants. These modifications differ from the traditional method in that a toxic component of required concentration is added to the bottles as a control. The experiment then determines primary production and destruction by the generally accepted method (Winberg, 1960). Investigations have shown that this test is not universal and may have an indicative value when dealing primarily with ions of heavy metals.

Under the action of heavy metals, the correlation of primary production and destruction is vastly altered. Copper, for example, influences both processes, while zinc increases destruction (Table 2).

In investigations of pollution of water by stable pesticides capable of inhibiting phytoplankton photosynthesis, the test in which the inten-

TABLE 2. PRIMARY PRODUCTION AND DESTRUCTION IN PHYTOPLANKTON SAMPLES (BLUE-GREEN + DIATOM) UNDER THE ACTION OF HEAVY METALS. VALUES INDICATED AS PERCENT OF THE CONTROL

Concentration of Ions, mg/l	Photosynthesis	Destruction
1.10^{-2}	10.6	38.8
5.10^{-1}	17.3	20.0
1.10^{-1}	4.2	11.1
1.10^{-3}	58.7	120.0
1.10^{-2}	116.0	180.0
1.10^{-1}	102.0	550
1.0	31.4	450.0

sity of gross photosynthesis is determined, may be a very distinct indicator of preservation of toxic substances in the water (Table 3).

In water containing herbicides, photosynthesis is inhibited in all algae, including the filamentous forms which may be used as indicators for visual detection of substances inhibiting photosynthesis. The test is performed in vessels with small lumps of filamentous algae (5-10 g wet weight), which, in a toxic medium, settles to the bottom, and does not become covered with bubbles of oxygen, but in the control they remain suspended in water and actively liberate oxygen. This test may be also performed on a glass slide with solitary filaments of *Cladophora*, or in a concave slide with a suspension of any planktonic algae. In both cases, the presence of toxic factor is indicated by the absence of formation of the bubbles of oxygen.

The most useful indicator organisms are aquatic invertebrates since they are more sensitive to toxicants than algae. Cladocerans, rotifers, larvae of mayflies and chironomids, garmarids, isopods, copepods, ostracods, bivalvia and gastropod molluscs are routinely used. Each of these organisms have their own specific features of behavior, biology, and reactions to toxic materials which must be taken into account when performing experiments.

TABLE 3. GROSS PHOTOSYNTHESIS IN A WATER-BODY TREATED WITH DIURONE AT THE TIME OF A BLUE-GREEN ALGAL BLOOM.

Days of Experiment	Temperature °C	Experiment (Diurone 0.2 mg/l)		Control
		O_2, Percent of Saturation	Photosynthesis mg/l hr^{-1}	O_2, Percent of Saturation
Background	21	111.8	12.0	224.2
1 hour after addition	28	57.2	0	-
1	23	0	0	124.5
3	25	0	0	152.8
5	23.5	0	0	165.2
7	23.0	0	0	71.8
10	23.0	0	0	66.0
12	19.5	0	0	109.8
15	17.0	0	0	154.0

In common tests on survival, the reaction to overwhelming intoxication is identical in all species, i.e., death. The percentage of mortality, for a given time may, however, vary significantly depending on the sensitivity of organisms, their anerobiotic ability, their lipid content, the degree of oxygen saturation of the medium, and many other factors. In addition to studies of mortality, visual behavioral reactions are also quite indicative. At present, experience in analysis of behavioral reactions in organisms is limited to two classes of toxicants, pesticides and heavy metals. It is possible that the reactions described are not universal, and the action of other toxicants is manifested in a different way. For example, the well known peculiarities of behavior of aquatic organisms in the presence of phenol (Alekseev, 1970) differs essentially from those described below for a number of species.

Cladocerans in a non-toxic medium move by leaps, rarely settling onto the bottom. The movements of the antennae are even. The heart rhythm in different species varies from 200 to 300 (occasionally up to 500) beats per minute, the eyes are brightly pigmented, and the body is multicolored. In a toxic medium the movements are predominantly rotatory and revolving around the body axis or along a spiral. The latter is especially characteristic reaction to toxic pesticides. As intoxication progresses, the crustaceans lie immobile, the body contracts convulsively, the antennae jerks, the heart beat diminishes to a single uneven contraction per minute, and the eyes become depigmented. The body may acquire a red coloration. The females abort immediately after transfer into the toxic medium, shedding both eggs and embryos. After prolonged exposures to low concentrations of toxicant, embryonic abnormalities may arise. These anomolies may include twisted antennae, underdeveloped eyes, and the like.

The copepods do not usually manifest symptoms of disturbed behavior in toxic media. The rotifers, however, pass into a state of anabiosis, change in body length, and cease to feed (observable microscopically by decoloration of the intestine). Counts of living, anabiotic, and dead rotifers in a Fuks-Rozental Counting Chamber or other hemocytometer establish the toxic effect quantitatively in comparison with the control.

In toxic media, the usually quick moving gammarids (*Gammarus pulex*, *G. lacustris*) become sluggish and nearly immobile. Under the influence of heavy metals, their bodies may acquire a red tint. Aquatic sowbugs react similiarly.

Chironomid larvae in water move in a spiral fashion. On a suitable substrate, they begin case construction. Under the influence of toxic substances, their body is convulsively stretched and straightened, they lie immovable on the bottom and fail to construct cases. In hemoglobin containing species (*Chironomus plumosus*, *C. semiredictus*) the red color may assume a greenish blue, or disappear altogether.

The image forms of chironomids appear to be rather sensitive to the presence of DDT residues in storage organs and tissues of fishes (Bereza, 1972). In contact with tissues of predatory fishes (fat, brain) containing considerable quantities of accumulated DDT, chironomids are paralized instantly, or they manifest clear symptoms of insectides poisoning: tremor of limbs and wings, disturbances of coordination, convulsions, and death in 20-30 min. after exposure. To check corresponding symptoms of intoxication and the rate of their development relative to the DDT content in tissues, the material was analyzed by the gas chromatographic method and scale of conventional values of the level of accumulation of DDT in tissues of fish is proposed (Table 4).

This test has a dignostic value when analyzing the causes of mass mortality of fish. Accumulation of DDT i.e. the vital organs of fish, especially in the brain, may be the cause of sudden catastrophic death in stress situations, e.g., after a sharp increase in water temperature, during spawning, and as a consequence of mobilization of fat reserves and resultant appearance of DDT dissolved in lipids in the blood streams. Detection of insecticides in the brain tissue of long preserved, or even petrified fish, enables identification of the role of DDT as one of the causes of death, while the chemical analysis of such tissue is very complicated, and requires equipment for gas chromatography.

The oligochaet *Tubifex tubifex* in non-toxic media normally maintains a vertical body attitude, swaying evenly like a wheat field in the wind. On the bottom, they form tangles. In toxic media the bodies of the worms stretch convulsively, the movements become disordered, and under deep intoxication, the worms lie immobile, unentangled on the bottom, and the reddish coloration of the body disappears (evidently as a result of hemoglobin degradation as in chironomid larvae).

TABLE 4. TESTS ON DDT CONTENT IN STORAGE TISSUES OF FISH

Species of Fish, Tested Tissue	Weight g	DDT Content (DDT+DDD+DDE) mg/kg	Toxic Effect On Image of Chironomids	Mark
Zander				
Intestinal fat	4.0	25.7	Instant death of all the insects.	++++
Liver	4.0	0.70	Clear symptoms of intoxication. Agony. Death in 20-40 min.	+++
Carp				
Liver	4.0	0.145	30-40 percent are dead in 1 hour.	++
Zander				
Muscle Tissue	4.0	0.03	Death in 1 hour.	+
Roach				
Muscle Tissue	2.0	0.00	All are living for 6-8 hours.	

In clean water, the molluscs *Anodonta*, *Unio*, and *Sphaerium* periodically open the valves and protrude both foot and syphons. In toxic media, the valves are either permanently closed or widely opened and the syphons are protruded. In the gastropods, the reactions to toxicants are quite diverse and peculiar. *Viviparus viviparus* in toxic median retracts the body deeply into its shell, tightly shuts the lid and becomes enveloped in a thick layer of musilage. The large pond snail, *Limnaea stagnalis*, actively grazes on plants and filters suspended particles in clean water, its body periodically protruding from its shell. The defensive reflex consisting of retracting the body into the shell as a result of external stimuli is clearly expressed in this mollusc. It normally produces a tape-like excrement, however, the water in the vessel remains clean and transparent, evidently owing to bactericidal effect of the excreted musilage and possibly to antibiotic substances. In toxic media, this mollusc does not feed, or the intensity of ingestion of food is greatly diminished, and the body is hidden in the shell. Other essential disturbances in behavior, notably in sexual behavior (Bereza, 1973), are evident. Under acute intoxication, this mollusc falls out of its shell.

The sensitivity of tests on survival of invertebrates may be considerably increased when experiments are conducted at elevated temperatures (Table 5). The sensitivity of biological tests is further increased with sharp changes in temperature.

When undertaking such testing, it is necessary to consider a number of factors, including: 1) hydrochemical peculiarities of water, its oxygen content, pH; 2) the degree of adaptation of the test organisms to the experimental conditions, their lipid content, age, sex, developmental stage; 3) temperature; and 4) the degree of pollution of the habitat.

However, in cases without significant statistical differences between the experiment and the control, and the test is of short duration, all of these conditions are of secondary importance, especially when drawing con-

TABLE 5. INCREASE IN SENSITIVITY OF BIOLOGICAL TESTS ON SURVIVAL OF AQUATIC ORGANISMS AT 30 °C

Toxicant	Test Organisms	Lethal Concentration, mg/l	
		at 18 °C	at 30 °C
$ZnSO_4 \cdot 7H_2O$	*Daphnia magna*	1.0	0.005
$CdSO_4$	(females with eggs)	0.2	0.002
$CuSO_4 \cdot 5H_2O$	*Daphnia magna*	0.02	0.009
$ZnSO_4 \cdot 7H_2O$	(young)	1.09	0.014
$ZnSO_4 \cdot 7H_2O$	*Asellus aquaticus*	39.0	1.52
$CdSO_4$	*Asellus aquaticus*	32.0	0.20

clusions about the toxicity of the water. Further evaluation requires special chemical analyses, toxicologic investigations, and a detailed ecological study of the water body.

The quantitative aspect of application of biological testing is more complex and requires an approach to the characteristics of measurable functions. Any function changing under chemical action may function as an indicator, although the more simply measured functions are advantageous to use. Various functional disturbances in highly organized aquatic organisms may be evaluated on the basis of application of various physiological and biochemical methods (Komarovski, 1971, 1972; Malyarevskaya and Birger, 1973).

At present, elementary statistical principles are being used in biological testing. However, it is impossible to avoid the influence of permanently acting factors (time, temperature) and yet, it is necessary to reduce expenditures of time and labor to a minimum. The most reasonable approach is the use of the principle of the All Factor Experiment (AFE). The scheme AFE 2^2 enables the acquisition of reliable data from four experiments. This seems applicable to the situation of stabilized temperature, with due consideration of the factors of concentrations, and time of action of toxicants. When three variable factors are considered, the scheme AFE 2^3 gives confident information using eight experiments. Such a material can be easily interpreted both analytically and graphically, using a system of three coordinates. With a mass accumulation of data an electronic computer can be utilized.

In conclusion, it should be noted that the development of the methodology leads to three variations of application: 1) qualitative tests for toxicity of medium, 2) quantitative characteristics of toxic effect, and 3) quantitative determination of toxicants. The latter task is the most complicated, and practically insoluble. In some cases, biological testing lacks sufficient sensitivity when compared with chemical analysis. For example, the test of Knepp compared with analytical chemical methods has shown that this test detects the presence of some toxicants only at concentrations equal to 5-10 MPC, which is certainly insufficient for a quantitative conclusion.

More indicative are the specific chemical tests, which reflect the effect of a given substance, and provide a general indication of the polluting substance. Concentrations are established by consecutive dilutions of the suspected toxicant. Parallel testing using a sensitive species is employed. The effective concentration corresponds to the dilution at which similar toxic effects are displayed. In view of the difficulties of identifying numerous substances in natural water, and the necessity for complex and expensive apparatus, often unsuitable for work in the field, the quantitative tests may not only have analytical value, but may also be more economic than direct determination of toxicants by chemical methods.

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SECTION 11

CHRONIC EFFECTS OF LOW LEVELS OF HYDROGEN SULFIDE ON FRESHWATER FISH

Lloyd L. Smith, Jr.

INTRODUCTION

The potential effects of hydrogen sulfide on fishery ecosystems have not been fully realized because most survey work has measured neither concentrations below 0.5 mg/l, nor accumulations near the sediment/water interface in areas of continuous H_2S production. Areas within a few centimeters of the bottom where fish eggs and young fry occur are rarely sampled. Since it has been assumed that levels of hydrogen sulfide potentially dangerous to fish life occur only under conditions of low oxygen concentration, it has been believed that the adverse effects of low oxygen will control fish populations before the toxic effects of sulfides will be manifested. Undissociated hydrogen sulfide is the toxic form. At pH 9.0, approximately 1 percent is undissociated, at 6.7 about 50 percent, and at 5.0 about 99 percent.

The significance of the work described in this study is primarily a demonstration of the toxic effect of very low concentrations of hydrogen sulfide which frequently are found over natural organic bottoms, in the vicinity of sludge beds, and in areas where hydrogen sulfide is formed from waste effluents or comes directly from industrial operations. Colby and Smith (1967) found levels of hydrogen sulfide within 20 mm of the bottom which varied from 0.02 to 0.20 mg/l in a river with a major walleye (*Stizostedion vitreum vitreum* Mitchell) fishery. Dissolved oxygen at these locations was adequate to maintain fish life. Here maintainance of the adult population depended on inward migration of fish rather than natural reproduction. In natural spawning areas for northern pike (*Esox lucius* Linnaeus), Adelman (1969) reported hydrogen sulfide concentrations near the bottom commonly in the range of 0.03-0.08 mg/l, and occasionally as high as 0.22 mg/l during the spawning period. Scidmore (1956) working in a Minnesota lake during the winter found 0.3 and 0.4 mg/l H S with 6.0 and 3.6 mg/l O , respectively. Adelman and Smith (1970) showed that eggs and fry of northern pike were affected by low levels of hydrogen sulfide.

The experiments summarized in the present report used four species of fish, brook trout (*Salvelinus fontinalis* Mitchill); bluegill (*Lepomis macrochirus* Rafinesque); fathead minnow (*Pimephales promelas* Rafinesque); and goldfish (*Carassius auratus* Linnaeus). The purpose of this study was to determine "no-effect" levels of hydrogen sulfide based on long-term tests, and to relate them to acute toxicity levels.

MATERIALS AND METHODS

The work described here was done at the University of Minnesota laboratories using well water (Table 1). Continuous flow-through apparatus was used in all tests. Small species and early life history stages were tested in equipment described by Colby and Smith (1967), and Mount and Brungs (1967). Adult fish of larger species were tested in fiberglass tanks and all other tests were done in glass or acrylic test chambers. Molecular hydrogen sulfide concentrations were maintained with sodium sulfide solutions, and pH was adjusted to provide the desired test concentration. Analyses of water from the center of each test chamber were made 3 times each day in acute tests and every 2 days in chronic tests. Using Method C of Standard Methods of Water Analysis (1971), undissociated hydrogen sulfide was determined by calculation. Bluegills and some fathead minnows were wild stock from local lakes or streams. Other fish were hatchery stock or laboratory reared. The LC_{50} values were calculated by standard methods using probit techniques.

Acute Toxicity

Acute tests were made with 4 to 5 concentrations of hydrogen sulfide arranged in a logarithmic series and one control. Temperature in various tests ranged from 6.1 °C to 26 °C depending on species, life history stages, and specific objectives (Table 2). Duration of tests was from 4 to 12 days to determine the 96-hr LC_{50} and the threshold LC_{50}. The threshold value was considered to be attained when no death occurred in 48 hours. Eggs, sac fry, swim-up fry, juveniles, and adults of fish species were tested. The LC_{50} at 96-hr varied from 0.515 mg/l H_2S at 6.1 °C with fathead minnow sub-adults, to 0.007 mg/l H_2S at 24 °C with fathead fry. Values ranged between these limits for other species, life history stages, and temperatures (Table 2).

The effect of temperature on resistance was large in fathead minnows. The 96-hr LC_{50} concentration increased from 0.021 mg/l at 24 °C to 0.515 mg/l at 6.1 °C (Table 2). This twenty-five fold change in tolerance occurred principally between 10 and 6.1 °C. Goldfish tested at temperatures between 14.1 and 26 °C showed 96-hr LC_{50} values varying from 0.145 mg/l H_2S at the lowest, to 0.063 mg/l H_2S at the highest temperature (Adelman and Smith, 1972).

Threshold LC_{50} concentrations were lower than those for 96-hr in most tests, but not markedly so, except in the goldfish at lower temperatures. Threshold values for the various life history stages had the same general relationships to each other as the 96-hr LC_{50} values.

Long-Term Tests at Low Concentrations

Since acute toxicity of a material may be a poor index of long-term effects of sub-acute concentrations of a toxicant on a fish population, a

TABLE 1. ANALYSIS OF WELL WATER[a] VALUES EXPRESSED IN MG/L

Item	Concentration
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	<0.0005
Cadmium, mercury	<0.0001
Ammonia nitrogen	0.20
Organic nitrogen	0.20

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[a] Water was taken from well head before aeration and heating; pH 7.5.

TABLE 2. 96-HOUR AND THRESHOLD LC_{50} OF HYDROGEN SULFIDE (MG/L) FOR BROOK TROUT, BLUEGILL, FATHEAD MINNOW AND GOLDFISH

Species	Stage	No. of Tests	Temp. °C	96-hr LC_{50} Mean (mg/l)	Threshold Mean (mg/l)	LC_{50} Days
Brook Trout	Egg	3	9.0	--	0.054	9
	Sac fry	2	9.0	0.031	0.030	5
	Swim-up fry	1	12.5	0.022	0.019	7
	Juvenile	2	8.0	0.025	0.019	12
Bluegill	Egg	2	22.0	0.140	--	--
	Sac fry	1	22.0	--	0.017	9
	Swim-up fry	1	22.0	0.009	0.008	8
	Juvenile	6	20.0	0.028	0.028	4
	Adult	8	20.0	0.030	0.030	4
Fathead	Egg	6	24.0	0.035	0.035	4-8
	Fry	3	24.0	0.007	0.006	6
	Sub-adult	4	6.1	0.515	--	--
	Sub-adult	1	10.0	0.150	--	--
	Sub-adult	1	15.0	0.057	--	--
	Sub-adult	7	20.0	0.036	--	--
	Sub-adult	6	24.0	0.021	--	--
Goldfish	Egg	1	22.1	0.022	--	--
	Fry	1	21.6	0.025	--	--
	Sub-adult	21	14.1	0.145	0.084	11
	Sub-adult	21	20.0	0.083	0.071	11
	Sub-adult	21	26.0	0.063	0.060	11

series of tests of extended duration at low levels of hydrogen sulfide were run on the test species (Table 3). An unfavorable response was assumed to occur when growth, survival, or reproduction were adversely effected. A "no-effect" concentration was identified when no adverse effect on these parameters was noted. Physiological responses may occur at these concentrations. In some species stimulation occurred at the lowest levels of treatment and resulted in better long-term performance than that exhibited by the controls.

Tests were conducted for 45 to 826 days in the various species (Table 3). The temperature at which various tests were run varied from 11.8 to 24 °C with different species. The "no-effect" concentration varied from a minimum concentration of <0.001 mg/l H_2S at 24 °C in bluegills, to 0.010 mg/l H_2S at 18.6 °C in one goldfish test. Bluegills were the most sensitive species. A comparison of trout with the warmwater species is difficult because the former were tested at lower temperatures. The life history stage at which different fish species were first subjected to hydrogen sulfide had varied influence on the final "no-effect" level in the different species. The concentrations designated as "lowest effect level" were the lowest concentrations of molecular H_2S which showed a measurable

TABLE 3. CHRONIC EFFECT OF SUBLETHAL CONCENTRATIONS OF HYDROGEN SULFIDE (MG/L) ON TROUT, BLUEGILL, FATHEAD MINNOW AND GOLDFISH

Species	Stage Started	Days	Temp. °C	No-Effect Level	Lowest Effect Level	Factor of Effect[3]
Brook	Adult	45-75[1]	12.9	<0.006	0.006	R
Trout	Fing. (5 g)	120	13.0	0.007	0.009	G
Bluegill	Eggs	316	22.4	--	0.002	G+S
	Juvenile	826	11.8	0.002[2]	0.003	G+S
	Juvenile	165	24.0	<0.001	0.002	G+S
	Adult	200	15.0	--	0.002	G
	Adult	288	20.2	0.003	0.006	G+S
	Adult	97	23.6	<0.001	0.001	R
Fathead	Eggs	84	23.0	0.005	0.007	G+S
	Eggs	404	24.0	0.004	0.007	G+S+R
	Juvenile	112	20.0	0.005	0.011	S
	Juvenile	373	21.3	0.007	0.019	G+S
Goldfish	Eggs	430	21.5	0.007	0.009	G+S
	Juvenile	294	18.6	0.010	0.025	G+S
	Adult	294	18.6	0.005	0.010	G+S

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[1] Two tests.
[2] Reproduction inhibition at 0.002 mg/liter.
[3] R, reproduction; G, growth; S, survival.

adverse effect. The two most useful indicators of adverse effects were growth and reproductive rates (Tables 4, 5, and 6).

Two experiments on bluegills were conducted, one started with young-of-the-year fish exposed for 826 days, and one with adults exposed for 97 days prior to spawning. At low H_2S levels, the percentage increment in weight after short exposure was greater than controls (Table 4). After 826 days exposure to 0.007 mg/l H_2S, the mean weight of the fish started as young-of-the-year was approximately 63 percent of controls. In a second test, after fish started as adults which were exposed for 97 days to levels of

TABLE 4. INCREMENT IN WEIGHT AND SURVIVAL OF BLUEGILL STARTED AS YOUNG-OF-THE-YEAR AND EXPOSED TO VARIED CONCENTRATIONS OF HYDROGEN SULFIDE FOR 826 DAYS AT O_2 OF 6.0 MG/LITER AND MEAN TEMPERATURE OF 17.8-18.5 °C VARIED SEASONALLY--WEIGHT EXPRESSED AS MEAN IN GRAMS AT SUCCEEDING INTERVALS

Exposure (Days)	H_2S Concentration (mg/liter)			
	Control	0.002	0.004	0.007
		Weight (g)		
56	4.02	4.50	5.00	5.12
392	50.81	46.34	34.37	43.35
826	99.91	98.71	90.35	63.05
		Percentage Survival		
28	100	100	90	100
362	100	100	90	100
392	100	100	90	100
420	100	100	90	100
826	100	100	90	70

TABLE 5. WEIGHT AND REPRODUCTION OF ADULT BLUEGILLS EXPOSED TO VARIED CONCENTRATIONS OF HYDROGEN SULFIDE FOR 97 DAYS AT 23.5-23.9 °C

	H_2S Concentration (mg/l)				
	Control	0.001	0.002	0.003	0.008
Mean Starting Weight (g)	75.9	84	78.2	76.1	76.6
% Increase in Weight	55	58	70	59	54
No. Eggs/Female	17,562	12,795	6,157	0	0
No. Eggs/g of Female	155.5	100.8	51.1	0	0

TABLE 6. GROWTH OF FATHEAD MINNOW IN 112-DAY EXPOSURE TO HYDROGEN SULFIDE AT 23 °C, pH 7.7, O_2 6.4-7.3 MG/LITER - EXPRESSED AS MEAN WEIGHT IN GRAMS OF THREE REPLICATIONS AT THE END OF SUCCEEDING 28-DAY PERIODS

	First Generation				
	Control	0.0004	0.0012	0.0031	0.0061
28 Days	0.106	0.111	0.116	0.106	0.115
56 Days	0.455	0.421	0.382	0.399	0.359
84 Days	0.685	0.688	0.595	0.714	0.610
112 Days[1]	1.234	1.114	1.066	1.180	1.097
Total % Increment	1,064	904	819	1,057	854
Survival %	97	90	93	93	50
No. Eggs/Female[2]	1,181	1,332	444	1,314	1,109
No. Spawning	92	80	30	60	24
Total Females	11	13	9	9	4

	Second Generation				
	Control	0.0007	0.0013	0.0037	0.0069
112 Days	0.616	0.387	0.649	0.533	0.391
140 Days	0.965	0.799	0.960	0.812	0.655
168 Days	1.249	1.167	1.222	1.079	0.830
252 Days	2.160	2.374	2.190	2.022	2.036
Total % Increment[3]	250	513	237	280	423
Survival %	97	93	90	90	64
No. Eggs/Female	912	1,130	413	535	799
No. Spawnings	116	101	88	39	30
No. Females	21	14	16	9	5

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[1] Start of spawning in first generation.
[2] After 297 days exposure to H_2S.
[3] After 404 days exposure to H_2S.

hydrogen sulfide up to 0.008 mg/l, there was no appreciable difference between treatments and controls, except at 0.002 mg/l where there was a significant increase in growth. Reproduction (number of eggs deposited per gram of female) in the second experiment was significantly reduced at 0.001 mg/l H_2S and completely inhibited at 0.003 and 0.008 mg/l H_2S (Table 5). In the experiment started with young-of-the-year no spawning occurred after 826 days at concentrations of 0.002 mg/l and higher. The failure of egg deposition appeared to be caused by the inhibition or absence of normal spawning behavior, since apparently average numbers of viable eggs for fish of comparable size were found in ovaries of non-spawning fish.

A two-generation, long-term experiment on fathead minnows (three replications) was also run to determine the "no-effect" levels of hydrogen sulfide. The test was started with eggs which were hatched and carried through to spawning adults. The second generation was continued with eggs from females reared in the same hydrogen sulfide test levels as the first generation. The first generation was continued for 297 days and the second for 404 days. Growth measurements were all made prior to the start of spawning in both generations. The day length was reduced and lengthened during the second generation to induce spawning, which resulted in a lengthening of the total exposure period. Hydrogen sulfide ranges in the first generation were 0.0004-0.0061 mg/l, 0.0007-0.0069 mg/l during the first 112 days of the second generation, and 0.007-0.0069 mg/l during the remainder of the cycle. For both cycles, mean temperature was 23 °C, pH 7.7, and O 6.4-7.3 mg/l (Table 6).

Growth in weight after 112 days in the first generation was less at all test levels. In the second generation, after 252-day exposure, there was an apparent growth stimulation at 0.0007 mg/ H_2S. Growth inhibition in the second generation occurred in the early periods, but was less in later periods, suggesting that early effects of exposure are greatest, and tend to lessen as growth proceeds. These results may be influenced to some degree by greater mortality of smaller fish in the hydrogen sulfide treatments. Survival was much lower than in the control at the highest treatment in both generations. In lower treatments, survival was also lower than the control, but not markedly so.

The success of spawning, measured by the number of eggs produced per female, did not appear to be affected in the first generation at any level of hydrogen sulfide treatment, although at the highest level, female survival was substantially lower than in the control. In the second generation, there was an apparent stimulation of egg production at 0.0007 mg/l, but a reduction at higher levels. The number at the highest level may have been increased by mortality of smaller females.

DISCUSSION

Both toxic and long-term toxic concentrations of hydrogen sulfide have been shown to be lower than levels commonly found in natural and polluted waters. Because sites of sustained hydrogen sulfide concentrations in the ecosystem are frequently overlooked, or low levels are not identified, the importance of this toxicant in potential fish producing waters is often not evaluated. Comparison of LC_{50} levels with those which have adverse effects after long exposure indicates that the 96-hr LC_{50} may be 3 to 8 times higher than the safe levels. The present work, and that of Smith and Oseid (1974), who examined the effect of hydrogen sulfide on early life history stages of 8 species of freshwater fish, show that a safe level of hydrogen sulfide which will insure survival and growth of a fish population, and adequate survival of all life history stages will generally be between 0.002 and 0.004 mg/l at 20 °C. In bluegills, the level is significantly lower, and this pattern may be followed in other species not tested.

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SECTION 12

THE BEHAVIORAL ASPECTS OF AQUATIC TOXICOLOGY

B.A. Flerov

As a result of conspicuous progress in the study of behavior of aquatic animals (Anon., 1972, 1975; Flerov, 1965), this science is becoming widely applied in aquatic toxicology.

As early as the 1950's, a number of manuscripts appeared demonstrating the peculiarities of action of pharmacological drugs by studies of the behavioral reaction of fish. These efforts (Abramson and Evans, 1954; Abramson *et al.*, 1958; and Evans *et al.*, 1956) were directed toward the study of the behavior of Siamese Fighting Fish (*Beta splendens*) under the influence of physycotropic drugs, including lysergic acid diethylanide, reserpine, and aminazine. Studies were also directed toward analysis of conditioned reflexes in fish exposed to the action of aminazine (Ivanova, 1961).

Later, evaluation of harmful effects of toxic substances were made using various behavioral reactions: general locomotory activity (Besch *et al.*, 1972; Waller and Carns, 1972), the character of swimming responses (Bengtsson, 1974; Davy *et al.*, 1972), feeding behavior (Braginski *et al.*, 1972; European Inland Fisheries Advisory Commission, 1965; Flerov, 1965; and Foster *et al.*, 1966), defensive behavior (Alekseev and Flerov, 1972; Flerov and Lapkina, 1976; Hansen *et al.*, 1972, 1973, 1974; Ishio, 1965, 1969; Jones, 1951, 1957, 1964; Sprague, 1971; Sprague and Drury, 1969), sexual behavior (Braginski *et al.*, 1972; Flerov, 1969), and choice of temperature optimum by aquatic animals (Ogilvie and Anderson, 1965; Opuszynski, 1971; Peterson, 1973).

The behavioral reactions serve as clear indices of the action of sublethal concentrations of toxicants. This is true not only of fish, but also of vertebrates (Swedmark *et al.*, 1971). At sublethal concentrations of surfactants, the authors note that behavior changed first in marine invertebrates; molluscs lost their ability to bury themselves into the sediments and closed the valves of their shells. Other authors (Alekseev and Flerov, 1972) have observed a suppression of negative photoreaction in chironomids and water mites under the action of sublethal concentrations of phenol. Braginski and Co-workers (1972) have established that in sublethal concentrations of hexachlorane, mating frequency in *Limnaea stagnalis* drops sharply. It follows from this, that the decline in population of molluscs may not be the result of the effects of hexachlorane on reproductive function, but a direct consequence of suppression of sexual behavior in the animals.

The changes in behavior of aquatic animals are not only clear indications of intoxication, they are also the first symptoms of disturbances of living activity. Just as an experienced physician can diagnose illness in his patient using only the patient's behavioral symptoms, a rather attentive toxicologist can speak of intoxication of an organism by judging its behavior. Therefore, behavior may be used as a sensitive test for toxicity of the water medium. In this regard, conditioned reflexive activity and learning of animals are of special interest.

Investigation of the conditioned reflexive reactions of fish under the influence of toxic substances have not achieved prominance until quite recently, in spite of the great experience gained by the Academician I.P. Pavlov and his followers on the pathophysiology of higher nervous activity under various generic pathogenic effects (Dolin, 1962; Frolov, 1944; Ivanov - Smolenski, 1952).

Investigations of the effect of phenol in sublethal concentrations upon locomotory defensive and locomotory feeding conditioned reflexes in gold carp (Flerov, 1965) have shown that the disturbances of the higher nervous activity of fish are of a general, nonspecific character, similar to pathological alterations in functioning of the cerebral cortex in mammals. These symptoms are manifested in inhibition of differentiation, decrease in percentage of demonstration of positive reflexes, prolongation of the latent periods of positive reflexes, and, finally, in complete suppression of conditioned reflex activity (Figure 1). The character and degree of disturbances of the reflexes depend upon the typological peculiarities of the higher nervous activity of a fish. In a fish of a weak type, pathological alterations begin earlier, and are displayed to the greatest degree (Flerov, 1973). Comparison of the sensitivity of the conditioned reflex method with other physiological methods has shown that it is order of magnitude more sensitive.

Alterations in the higher nervous activity may be successfully used as a quality sensitivity test for determination of the quality of water. The most varied methods of investigation of the conditioned reflex activity may be applied for this purpose. In recent time this methodology is being used to estimate the effects of low concentrations of mineral oil (Kasymov and Rustamova, 1969), heavy metal ions (Krasnov, 1971; Wier and Hine, 1970), and pesticides (Anderson and Peterson, 1969; Anderson and Prins, 1970; Hatfield and Johansen, 1972; McNicholl and MacKay, 1975).

Water toxicologists have long been using disturbances of equilibrium reflex as an index of intoxication in fish. Little attention, however, has been paid to careful observations of all the symptoms of intoxication, and to their objective recording.

Two examples illustrate this observation. The first example considers the symptoms of acute intoxication in fish as a result of exposure to

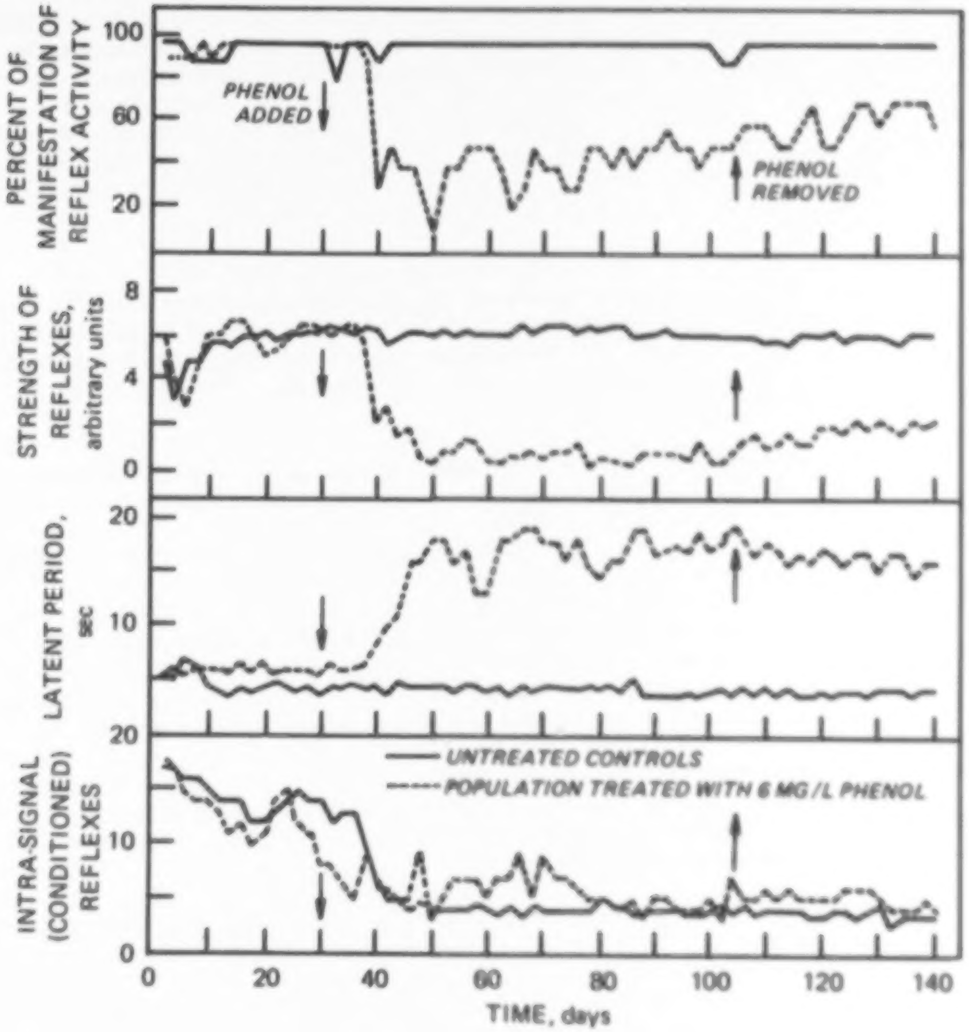

Figure 1. Changes in the conditioned reflex activity in the common guppy (*Lebistes reticulatus*) under the influence of sublethal concentrations of phenol.

several different classes of toxic compounds (Table 1). In spite of the fact that there are common features in the manifestation of pathology (e.g., violent general locomotory activity, disturbance of equilibrium reflex on intoxication with phenol and polychlorpinene), characteristic specific features are also revealed. Thus, under the influence of polychlorpinene, in contrast to phenol, such specific symptoms as moving to the surface and swallowing of the air are observed in fish during the phase of the violent locomotory activity.

On intoxication with chlorophos, a prolonged inhibition stage and darkening of coloration due to the opening of chromatophores are characteristic. An abundant excretion of mucilage is observed as a result of exposure to detergents.

A second example is to be found in the symptoms of intoxication in one of the representatives of invertebrates, the medicine leech (*Hirudo medicinalis*: Annelida). Exposure of this organism to solutions of the toxic substances noted above yields more specific reactions (Figure 2).

Intoxication with polychloropinene is first noted when the organism rolls the anterior body segments ventrally (Figure 2, 1-4). A few hours later, convulsions develop followed by immobility and death.

TABLE 1. SYMPTOMS OF INTOXICATION IN CARP EXPOSED TO THE SHORT-TERM ACTION OF TOXIC SUBSTANCES

Toxicants	Symptoms of Intoxication
Phenol	Violent locomotory activity (fish perform impetuous rushes, frequently breaking their snout against the walls of aquarium). Disturbance of the equilibrium reflex (swimming on the side); convulsions; immobilization; death.
Polychlorpinene	Prolonged increase in general exitability to acoustic and tactile stimuli, violent swimming activity. Fish swims to the surface and swallows the air. Disturbance of equilibrium reflex; immobilization; fish floats up and dies.
Chlorophos	Increase in general exitability to acoustic and tactile stimuli. Sudden general inhibition (weak reactions to external stimuli, low general activity); disturbance of coordination; tremor of body muscles; significant intensification of coloration; sinking of the fish to the bottom; immobilization; death.
"Lotos-71"	Abundant excretion of mucilage; immobilization; death.

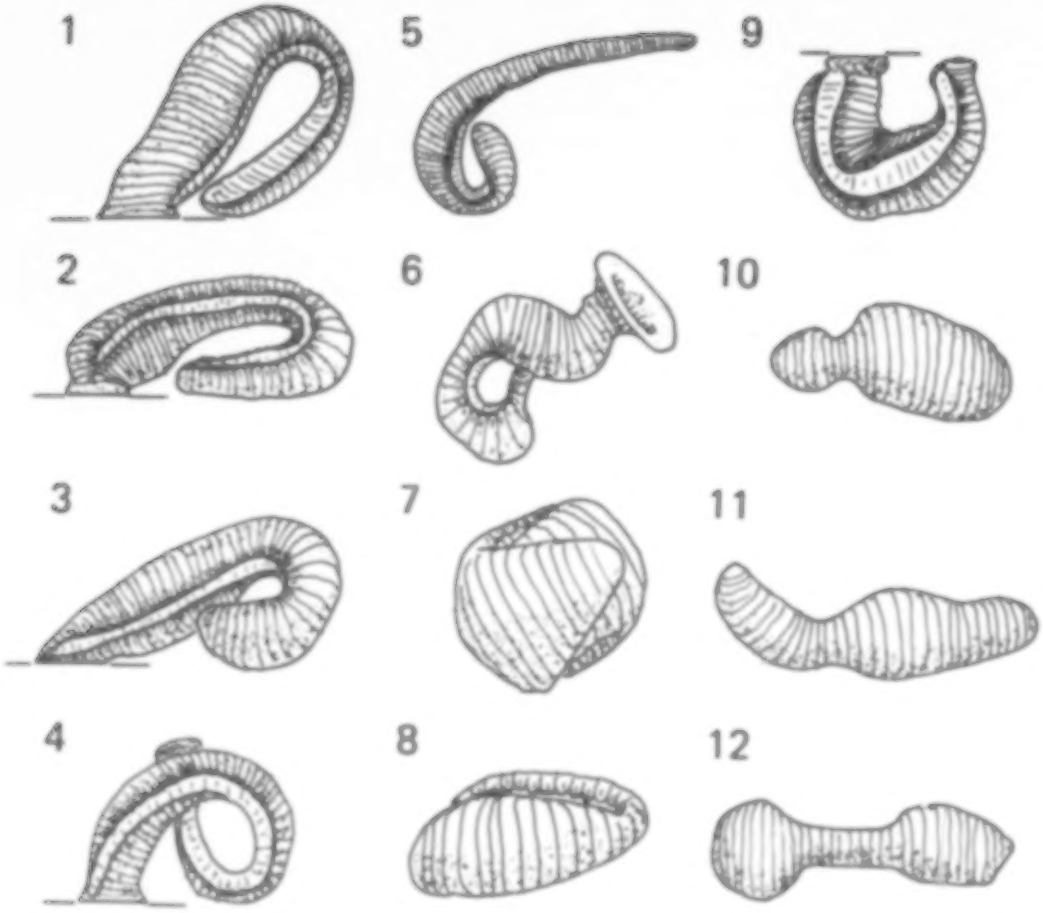
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Figure 2. Symptoms of intoxication of medicine leech in solutions of polychlorpinene (1-4), chlorophos (5-8), and phenol (9-12). See text for explanations.

In solutions of chlorophos, initial symptomology is manifested by coiling of posterior segments, and twisting them into a spiral. Gradually, twisting of the whole body into a tight spiral occurs, so that the movement ceases (Figure 2, 5-7). The leech subsequently straightens, the gullet opens and swallowing of air occurs. Both volume and weight of the organism increases (Figure 2, 8).

In a solution of phenol, initial disorderly locomotor activity and coordinative disturbances are followed by a looped attachment to the walls of the vessel (Figure 2, 9). The leech then drops to the bottom, convulsions develop and characteristic constrictions appear in the body (Figure 2, 10-12). Such observations enable an estimation of the character and specificity of action of a harmful compound, suggest a course of study of actual functions responsible for the development of the pathological process, and enable classification of the intoxications.

The most important problem presently facing aquatic toxicology is the question of adaptation of the organisms to a new environmental factor, the toxicants. Because of their active relationship with the environment, aquatic animals can avoid the harmful effects of such factors, though defensive behavior incorporating the avoidance reaction.

The ability of fish to avoid toxic solutions under laboratory conditions has been widely considered (European Inland Fisheries Commission, 1965; Hansen *et al.*, 1972, 1974; Ishio, 1965, 1969; Jones, 1951, 1957, 1964; Shelford, 1971; Sprague and Drury, 1969). The experiments have been performed using many species of fish (carp, crucian carp, minnow, loach, trout, etc.) and various toxic compounds: cyanids, phenols, salts of zinc and copper, carbonic acid, ammonium, chlorine, hydrogen sulphide, pesticides, detergents, and industrial wastes. In general, the results of the experiments have shown that various species of fish avoid the zones with sublethal concentrations of toxic substances.

Ishio suggests that the avoidance reaction obeys the law of Weber-Phekhner, i.e., the response is proportional to the logarithm of the irritant intensity (concentration of the toxic substance). However, this reaction varies widely, depending on the species of fish, and the chemical properties of the toxicants. Its manifestion may be strong, weak, or entirely absent. For example, phenol is actively avoided by the carp, but the contaminant is not avoided, even in lethal concentrations, by salmonids. Moreover, some toxic substances are even preferred by fish. Thus, low alkalinity, ammonium hydroxide, and low concentrations of copper salts possess attracting properties. The reaction of trout to dissolved chlorine is most interesting (Sprague and Drury, 1969). A distinct avoidance reaction is observed in the range of low (0.001-0.01 mg/l) and high (1 mg/l) concentrations, but in the range of medium concentrations, a clear preference is revealed. It is difficult to explain the difference in reactions. Studies of associated physiological mechanisms are still lacking. However, perception and discerning of toxic substances is largely provided by organs of smell and taste, and to the so-called organ of general chemical sense. For example, fish can discern phenol and parachlorphenol in concentrations as low as 0.0005 mg/l (Hasler and Wisby, 1950).

There are still few data on the avoidance reaction to toxic substances in aquatic invertebrates. Studies have utilized freshwater insects, spiders, leeches, and marine crustaceans. The forms of behavior preventing toxic exposure to animals are varied. Beetles usually crawl out of toxic solutions along the walls of the experimental vessels and occasionally even fly out of the solutions. Hymenoptera jump out and fly away, and water spiders escape by running along bits of grass placed into the vessel. Animals incapable of rapid movement manifest defensive behavior in other ways. The larvae of many flies and some butterflies use their cases as means of protection from toxicants. Some caddis fly larvae in toxic media will build thicker case walls. Chironomids bury themselves deeper into the mud. Molluscs close the valves of their shells tighter and for a longer period (Alekseev and Flerov, 1972).

The most dangerous toxic substances (pesticides) are, however, poorly avoided by invertebrates. Again, the medicinal leech will serve to illustrate this fact. A comparison of avoidance of substances belonging to different classes of chemical toxicants (Table 2) shows that pesticides are either not avoided at all (chlorophos), or avoided only at their lethal concentrations (polychlorpinene). These substances are evidently not "unpleasant", causing pain for the leech. Herein lies the insidiousness of pesticides. In contrast, phenol and "Lotos-71" were actively avoided by the animals.

The avoidance reactions are of great importance for adaptation. For practical purposes, it is essential to know the range of concentrations in which these reactions are manifested. However, questions arise relative to the extrapolation of experimental results into the natural system.

There are some observations on migrations of marine fish from the areas polluted with petroleum wastes. Data also exists on impoverishment of species composition of communities which may be explained, not only by the death of some species, but also by escape of others from the polluted

TABLE 2. TOXICITY OF SOME SUBSTANCES FOR MEDICINE LEECH AND THEIR THRESHOLD CONCENTRATIONS (MG/L) PRODUCING AVOIDANCE REACTION

	Exposure 48 hrs.			
Toxicants	Maximum Tolerated Concentrations	LC_{50}	LC_{100}	Threshold Concentration
"Lotos-71"	150	190	300	1
Phenol	275	290	100	50
Polychlorpinene	2.5	5	10	5
Chlorophos	0.05	0.3	0.6	No avoidance

areas. The initial comparison of avoidance reactions in the laboratory and in the field yields barely consoling results. Mature salmon actively avoiding salts of copper and zinc under experimental conditions displayed virtually unnoticeable reactions in nature (Sprague, 1971). Studies of this kind are very important, and their further development is required.

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SECTION 13

GEOLOGIC POLLUTION PROBLEMS OF LAKE SUPERIOR

Albert B. Dickas, Ph.D.

ASBESTIFORM MINERALS

Background

On 17 February, 1972, the U.S. Environmental Protection Agency first stated that the presence of the trace mineral cummingtonite in the Wisconsin portion of Lake Superior constituted interstate pollution. This statement was made part of preliminary evidence being gathered in the now famous "Reserve Mining Case" (Reserve Mining Company versus United States of America, No. 74-1291). In December of that same year the first public statement was made of concern over the relationship of the presence of asbestos and potential public health hazards of residents of the Lake Superior Basin (Great Lakes Research Advisory Board, International Joint Commission, 1975). Since then a number of detailed investigations of the distribution and health effects of asbestos have been undertaken.

Prior to this time, the region was internationally known as an economic source of silver (historically), copper, as well as high grade (hematite) and low grade (taconite) iron ore, but not asbestos in any commercial quantity. Thus little research has been done on the distribution of this mineral within the Great Lakes area. Although the effects of inhaled asbestos are reasonably well known to be the causative agent of the disease asbestosis (a scaring of the lungs by increased fibrous tissue growth), the effects of ingested asbestos have only recently been considered by the scientific and medical community.

Mineralogy, Chemistry and Morphology

The term asbestos is defined by Dana (1954) as constituting the fibrous varieties of serpentine and amphibole, the fibers of which are sometimes very long, fine, flexible, easily separable by the finger and look like flax. The term is derived from the Greek for "incombustible". Generically, the term is used to describe fibrous hydrated silicates, consisting of 40-60 percent silica in combination with oxides of iron, magnesium and other metals. The minerals differ in their chemical and physical properties, such as fiber diameter, flexibility, tensile strength and surface properties.

A conventional description would include highly perfect cleavage, subconchoidal to uneven fracture, vitreous to pearly luster, black, white, green, brown and pink in color and with an uncolored streak. Pleochroism is strong, with deeply colored varieties with absorption usually $Z>Y>X$ (Dana, 1954).

Mineralogically there are two main groups of asbestos minerals: serpentine and amphibole. Common types, crystal class, chemistry and identification characteristics are given in Table 1.

Water is considered an essential constituent of all types. Common fiber lengths for all of the asbestos minerals are within the range of 0.2-10 μm (μm = 10^{-6} meter). These values depend somewhat on the laboratory method employed to disperse the fibers for measurement.

For bulk analysis, the techniques of infrared spectroscopy, differential thermal analysis and X-ray diffraction are employed. In order to study individual fibers, transmission electron microscopy, selected area electron differaction and electron microprobe analysis methodology are employed. While acceptance precision can be achieved through these expensive and time-consuming programs, the superiority of any one method has yet to be demonstrated (Great Lakes Research Advisory Board, International Joint Commission, 1975).

Geological Sources

Chrysotile asbestos occurs in serpentine form that has been altered from (a) ultrabasic rocks such as peridotite or dunite or (b) magnesium limestones or dolomites (Bateman, 1950). The former occurrence yields about 90 percent of the world's asbestos supply. Amphibole varieties are found in slates, schists, banded ironstones and as lenses and pockets in peridotite and pyroxenite.

TABLE 1. CLASSIFICATION OF ASBESTOS. DATA FROM GREAT LAKES RESEARCH ADVISORY BOARD, INTERNATIONAL JOINT COMMISSION, 1975

Group	Name	Chemistry	Crystal	Characteristics	
Serpentine	Chrysotile	$[(Mg,Fe)_2SiO_4]$	Monoclinic	Hollow Curved Fibers: OD $\approx$ 250 Å; ID $\approx$ 50 Å; often occur in bundles and change shape in lung liquids.	
Amphibole	Anthophyllite	$[(Mg,Fe)SiO_3]$	Orthorhombic	Straight: do not occur in bundles and do not split or change shape in lung liquids	OD>2500 Å
Amphibole	Cummingtonite*	$[(Mg,Fe)SiO_3]$	Monoclinic		OD>1500 Å
Amphibole	Grunerite*	$[(Fe,Mg)SiO_3]$	Monoclinic		OD>1500 Å
Amphibole	Tremolite**	$[(Ca,Mg)Si_4O_{11}]$	Monoclinic		OD> 600 Å

*That particular amphibole generally released into Lake Superior by Reserve Mining Company tailings discharge of 67,000 tons/day.

**That particular amphibole found in natural lacustrine sediments of Lake Superior.

Generally speaking asbestos might be found wherever basic and ultrabasic rocks have been serpentinized (the conversion of ferromagnesium minerals or rocks to aggregates of serpentine minerals) by autometamorphism (metamorphism of igneous rock by its own volatile fluids) (Bayly, 1968), thus forming chrysotile; or by load metamorphism (deep burial accompanied by mineralizing vapors), thus forming amphibole asbestos. This brief description of asbestos sources and formation fits quite nicely the geological conditions found within the Lake Superior Basin.

Source

The history of taconite mining in the Lake Superior Basin is the history of low grade iron ore beneficiation which resulted from the effective exhaustion of primary high grade hematite ores. Taconite is well cemented, ferruginous chert and slate. In order to be useful, such rocks must be beneficiated from material containing 25-30 percent iron to material containing as much as 65 percent iron. This high grading is accomplished by crushing and grinding the taconite to the point where in excess of 90 percent of the material is finer than flour. The purpose is to isolate the iron "ore" from the silica "gangue", so that the ore can be separated by a magnetic process.

A large quantity of water is employed in this process, both for continued washing and sizing of the material, and as a medium for handling. In the beneficiation process, the iron "flour" is made into "green" pellets, approximately 1.27 centimeters (0.5 inches) in diameter by rolling a mixture of bentonite clay and magnetic grains in a large revolving drum. The "green" pellets are then baked to 1316 °C (2400 °F) in a kiln where they are converted to taconite pellets, a very desirable blast furnance feed. In the process, up to 37,850 liters (10,000 gallons) of water are used for each ton of pellets produced. In addition, the ratio of waste tailings to concentrated pellet production is approximately 2/1, that is, two million tons of tailings to 1 million tons of pellets (Great Lakes Research Advisory Board, International Joint Commission, 1975).

Pollution problems associated with this beneficiation process relate to the dumping of tailings into Lake Superior and the voluminous use of water, the state in which it is left, and where the waste is disposed. All are concerns of the health and environmental community.

Treatment

Treatment of asbestos containing waters falls into two principle methods:

1. Ordinary filtration by sand or diatomaceous earth, which has proven to be approximately 90 percent effective.

2. Chemical coagulation with iron salts and polyelectrolytes followed by filtration, which is more than 99 percent effective.

Possible Health Effects

Although the effects of inhaled asbestos are reasonably well documented, the effects of ingested asbestos have only recently come under study. The Great Lakes Research Advisory Board indicates the following occupational hazards:

1. Asbestosis; increased fibrous tissue growth of the lung. Disease will appear after 10-40 years of occupational exposure.

2. Pleural calcification; a deposition of insoluble salts in the lung lining. Occurs after an approximate 20 year latency period.

3. Mesothelioma of the chest and abdominal cavity lining; with a latency period of 20-40 years, this disease was considered, until recently, quite rare.

Much less is known regarding ingested asbestos. It has been shown that the high rate of stomach cancer in Japanese is linked to their use of rice dusted with asbestositic talc (Merliss, 1971). Laboratory tests employing rats show that asbestos will accumulate in the brain and in tissue surrounding the small intestine. It may also cause malignant tumors in the kidney, lymph-nodes and brain (Pontefract and Cunningham, 1973). However, present knowledge of public health aspects of asbestos in drinking water supplies is inadequate. In consideration of the potential of this problem, the significance of a possible 20 year delay following even short-term exposure must be given proper perspective (U.S. Circuit Court of Appeals, 1974).

Considering direct biochemical effects to Lake Superior, taconite tailings deposition has shown to bring about the following results (Federal Water Pollution Control Administration, 1970):

1. A reduction in the abundance of fish food production sufficient to create five (5) percent reduction in commercial and sport fishing.

2. Chemical analysis projections, based upon daily discharge of 67,000 tons of tailings, indicates daily discharge of copper, nickel, zinc, lead, chromium, phosphorus and manganese ranging from 1,860 kilograms to 285,310 kilograms (4,100 to 629,000 pounds).

RED CLAY TURBIDITY

Based on the discharge analysis of major tributary systems, open lake turbidity and shoreline recession rates, it is estimated that the total gross erosion into the U.S. portion of Lake Superior is in excess of 4.8 million tons/yr. (a literature review of this subject will yield a wide estimate due to obvious field sampling difficulties and variability of discharge rates and storms with time of season). Along the north shore of the lake the estimated average annual yield is 1.1 ton/km^2, a relatively low figure due to geology (basically igneous and Precambrian strata), soil types, vegetation and land use. Along the south shore, where Pleistocene lake development sequences left a thick, exposed layer of easily erodible red clay, estimates range in excess of 6.8 ton/km^2 (Great Lakes Basin Commission, 1975). Thus, the subject of turbidity is basically one of red clay erosion along the Lake Superior south shore.

Of particular concern from the viewpoint of sediment erosion control has been the question of ultimate source of such lake turbidity: tributary inflow, lake shore erosion by high water levels, storm activity, and resuspension of previously deposited clay. The latter source is of significant debate considering the circulation patterns of Lake Superior. While the inflow and outflow rate of this lake is small in comparison to the water mass, the lake water is not standing still. It is kept in constant motion principally by the wind, which not only generates the visible surface waves (in excess of 4.9 meters), but stirs and mixes the water throughout the lake. Both water movements and rates of mixing are influenced by the formation of temperature (and associated density) thermoclines. In the summer, Lake Superior becomes divided into an upper layer of warm readily circulating water, the epilimnion, and the lower layer of cold, relatively undisturbed water, the hypolimnion. The contact between these two zones where rapid temperature changes takes place is termed the thermocline. When the lake is stratified, the hypolimnion is essentially physically and chemically isolated from the remaining water. In Lake Superior, nearly 95 percent of the lake's volume is in the hypolimnion (Federal Water Pollution Control Administration, 1969). The summer stratification begins to develop in mid-July, with the epilimnion reaching its maximum temperature of approximately 21 °C in August. In the winter months, the lake can be considered, for all practical purposes, to be isothermal.

Because currents in the lake are motivated principally by wind, and the winds are variable, the horizontal movements of lake waters exhibit infinite variety, and frequent changes in both direction and speed. The net circulation is counter clockwise, with the possibility of large cyclonic eddies occurring in the western arm (Great Lakes Basin Commission, 1975). Upwelling occurs in the lake when winds cause horizontal surface movement of water away from the shore and the surface waters are replaced by colder, deeper water (Upper Lakes Reference Group, International Joint Commission, 1977).

Considering such currents, storms, and precipitation cycles, it has recently been estimated that lake turbidity is primarily due to shoreline erosion of lacustrine clay by storm currents. The most recent available data (Sydor, 1975) indicates rates for the three causes to be:

1. Tributary erosion into Lake Superior: >500,000 metric tons per year

2. Shoreline erosion into Lake Superior: > 4,000,000 metric tons per year

3. Resuspension of lacustrine clay in Lake Superior: >300,000 metric tons per year for depths of $\leq$ 21 meters ($\leq$ 70 feet).

Chemical Effects

The water quality of western Lake Superior is directly affected by extensive erosion of the glacial-lacustrine red clay deposits (Dikas *et al.*, 1973). In addition to loss of property value, increased turbidity in drinking water, and a decrease in aesthetic value, Bahnick (1976) has attributed the following aquatic chemical changes to red clay turbidity.

Parameter	Turbid Conditions	Deep Water Conditions
Suspended Solids	>110 ppm	∼ 0.5 ppm
Alkalinity (ppm $CaCO_3$)	> 42 ppm	∼40 ppm
Hardness (ppm $CaCO_3$)^3	> 50 ppm	∼46 ppm
Calcium	> 15 ppm	∼14 ppm
Magnesium	> 3 ppm	∼ 2.7 ppm
Sodium	> 1.5 ppm	∼ 1.3 ppm
Iron	> 19 ppm	∼ 4.0 ppm
Orthophosphate	> 0.04 ppm	∼ 0.02 ppm
Chemical Oxygen Demand (Mg O/1)	> 16 ppm	∼ 3 ppm

Biotic Effects

In addition to human health effects, the problems of biotic effects of red clay turbidity centers on the overall effect upon the fisheries resource of Lake Superior. Recent studies have approached this problem from the viewpoint of turbidity effects upon species composition, feeding, predation, distribution, mortality and growth. Swenson (1975) has found the following preliminary results:

1. Changes in food habits of major species can be expected to result indirectly from turbidity through its influence on light penetration, fish distribution and distribution of plankton suites. Increased predation by pelagic smelt on larval herring as a result of turbidity may have resulted in the decline of commercial lake herring population as smelt can be expected to leave the bottom during turbid periods and increase predation pressure on the herring larval.

2. Turbidity may have indirect effects on walleye feeding success, rates and time.

3. Tank experiments indicate lake trout have a preference for low turbidity, while walleye showed a demonstrated preference for the highest turbidity levels.

In addition, red clay deposition would be expected to clog potential spawning grounds in the shelf areas of the lake. Finally, on a more qualitative basis, some Lake Superior commercial fishermen claim that if the clay material is readily visible in suspension during the time of freeze-up, then the winter fishing catch is greatly reduced.

This subject is a very complex one and continues to be studied in the Lake Superior Basin.

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SECTION 14

EXPERIMENTAL APPLICATION OF VARIOUS SYSTEMS OF BIOLOGICAL INDICATION OF WATER POLLUTION

G.G. Winberg

In the Soviet Union, there is no generally accepted system of evaluation of water pollution by hydrobiological indices. As in a number of the European countries, the most widely utilized was the saprobian system of Kolkwitz and Marson particularly modified by Zelinka and Marvan, Pantle and Buck, and Sladecek. At present, work has been started on the relative evaluation of various methods of biological analyses of water pollution. It should be noted that hydrobiological analysis may be used for two essentially different purposes: 1) to obtain a relative evaluation of the water quality at as given time, and 2) to obtain data objectively characterizing the condition of aquatic ecosystem, intended to be used subsequently for the study of long-term alterations.

Comparative evaluation of various systems of hydrobiological analysis of polluted waters has been made by the staff of the Laboratory of Freshwater and Experimental Hydrobiology of the Zoological Institute of the USSR Academy of Sciences. For this purpose during 1973-1975, hydrobiological samples were collected at 26 stations representing various degrees of pollution on several rivers of the Leningradskaya region, including the rivers: Izhora, Luga, Vuoksa. In the Kaliningradskaya region the tributaries of the system of the Pregol were included as was Moskva River. On all the rivers, the samples were collected in July and August of 1973-75 with the exception of the Izhora River, which was considered as a model and where the samples were taken on eight occasions representing all the seasons of the year. Detailed investigations were made of the phytoplankton and periphyton (V.N. Nikulina), planktonic ciliates (T.V. Khlebovitsch), zooplankton (M.B. Ivanova, L.A. Kutikova, A.V. Makrushin) and zoobenthos (A.F. Alimov, N.P. Finogenova, E.V. Balushkina, S.Ya. Tsekholikhin). The degree of pollution on each of the stations was characterized by hydrochemical (N.G. Ozeretskovskaya, V.V. Bulion) and bacteriological (M.F. Fursenko) data. Total counts of bacteria were made, the number of heterotrophic bacteria (plate counts on MPA) and the associated heterotrophic activity were determined by the method of Right and Hobbie. Of the 6 classes of polluted waters, only the classes II-V were represented, i.e., very clean (class I) and very dirty (class VI) water were not found.

Application of various modifications of the method of saprobic indicator organisms (including the methods of Knepp, Pantle and Buck, Zelinka and Marvan, Rotshain, Sladecek) was made to collections of phytoplankton, planktonic ciliates, rotifers and crustaceans. The results lead to very similar evaluations of the quality of the waters.

Detailed algological investigations on the 5 rivers showed that 50 percent of the species observed and nearly 80 percent of the dominating species are cited in the lists of saprobic indicators by Sladecek. This enabled a comparison of the methods of treating the data by Knepp, Zelinka and Marvan, Rotshine and Sladecek. The greatest possibility of differentiation of the stations with varying degrees of pollution are provided by the estimation of the mean saprobic valency according to Zelinka and Marvan. This method, while useful, can not compensate for the advantages of simplier methods which generally lead to similar results. This is especially true of the method of Sladecek, or to be more correct, his modification of the method of Pantle and Buck.

All the 5 investigated rivers must be classified as β mesosaprobic waters in spite of evident differences in their degree of pollution according to the saprobic index. Within this class, however, the rivers are arranged in a sequence by ranking the mean saprobic index corresponding to relative pollution.

Of 120 species of planktonic crustaceans and rotifers, 97 are included in the list of Sladecek. Estimation of the saprobic index lead to the same general conclusions as were drawn by data available on the phycology. The methods based on the application of the lists of saprobic indicators of phytoplankton, planktonic crustaceans and rotifers generally reflected the correct varying degrees of pollution of the investigated rivers, but they displayed only poorly the differences between stations on the same stream, especially when the influence pollution levels were low. This fact naturally established boundaries for the application of these methods.

The results of the zoobenthic assays lead to other conclusions relative to the application of the system of saprobic indicator organisms. The proposed systems of indicator organisms appeared to be not applicable in many cases for conditions in the USSR. One of the reasons is the difference between the fauna of the Middle European countries, and the rivers of the USSR. For example, 132 species of macrobenthic forms are presented in the table of indicator organisms. Of 170 species of benthic animals from our collections, only 17 can be found in the table of indicator organisms. The average number indicator species in a sample usually did not exceed 28 percent. At some stations on the Moskva and Vuoksa Rivers, the indicator organisms in the zoobenthos were absent entirely. The difference between the fauna and the number of the indicator species will be even greater for the areas of the Far East, Kamchatka, Sakhalin, Middle Asia, the Caucases and the like.

It is difficult to agree with those values for the saprobic valencies and indicator weights which Zelinka and Marvan give for *Tubifex Tubifex*, *Limnodrilus hoffmeisteri*, and *Potamothrix moldaviensis*. The first two species are indicated as characteric of α and p saprobic zones, and the latter only for α saprobic zones. At the same time, it is well known that all of them are typical also of β saprobic zone, and T. *tubifex* is one of the primary species of oligochaets in oligotrophic lakes.

Other varieties of the system of Kolkwitz and Marson (Knepp, Pantle and Buck) contain arbitrary evaluations of the number of organisms. These do not seem to be sufficiently correct to be applied to benthic animals. The use of the terms "many" "few" in these systems for quantifying organism abundance will have a variable meaning and cannot be applied with certainty.

Of current wide use for the evaluation of the degree of pollution is a system based on the application of large numbers of benthic taxa. It has been evident for some time that groups of aquatic insect larvae occur in clean waters. Oligochaets, on the other hand, easily resist pollutions and attain great abundance in the sediments enriched with organic matter. Therefore, it is not surprising that indices which account for abundance or biomass of oligochaets, or their separate species (Parele, 1975; Carr and Hiltunen, 1965; Goodnight and Whitley, 1961; Zahner, 1964, and 1965), or compare the ratio of the biomass of insects to that of oligochaets (King and Ball, 1964) are the most widely occurring.

Evaluation of the degree of river pollution with these indices has shown that some of them are probably correct only for those water-bodies for which they were proposed (American Great Lakes, Boden Lake, the Daugava and the Lielupe Rivers). The index of King and Ball does not account for the seasonal dynamics associated with numbers of insect larvae. Thus, one time collections may lead to incorrect values. The index which deserves attention is the one suggested by Zahner which considers the number of oligochaets, T. *tubifex* and species of the genus *Limnodrilus*. In this system, seasonal dynamics of oligochaets are considered in quite an unusual way.

The method with the greatest perspective for biological analysis of polluted waters using the composition of the bottom animals seems to be the one proposed by Woodiwiss (1964) for the Trent River. The undisputed advantage of this method is that it unites the principles of indicator values of separate taxa (distinctly fewer than the indicator species list), and the principle of decreases in diversity of the fauna under the conditions of pollution. It is important that Woodiwiss' system of "grouping" is understood to be rather broad. For some animals this implies separate species (larvae Plecoptera, Ephemeroptera), for others it suggests large taxa (e.g., the famely Tubificides). At the same time, this system reflects a simplification of trophic relationships with respect to pollution, e.g., the decrease in the numbers or disappearance of predatory animals.

Having evaluated the data by Woodiwiss' method, stations with a biotic index 7-9 were placed into the category of clean waters, 5-6 into moderately polluted, 4 was considered polluted, and 2-3 indicative of dirty waters. Extreme gradations, especially clean (Index 10) and very dirty waters (Index 0-1) were not encountered. This evaluation of the degree of pollution reflects rather objectively the actual situation in parts of the rivers investigated. The calculated values of the biotic index showed a good correlation with such chemical indices of pollution as BOD_5, and biochromate oxygen consumption (Figures 1 and 2). These figures show that the value of the index regularly decreases with an increase in BOD_5 and COD.

It is especially important that such an essential factor for distribution of benthic animals as bottom type did not interfere with the evaluation of the degree of pollution when using Woodiwiss' technique. Thus, samples taken on the cleanest station on the Izhora from silt gave the same high index value of 5 as those taken from stones on the same station. Samples taken from the same bottom types, subject to different degrees of pollution, had different values of this index. For example, samples taken from stones in various parts of the stream had indices of 4 and 9; from clean sands, 2.5, 7; from silted sands, 2-6; and from silts 2-5. This provides an assurance that the values depended primarily on the degree of pollution. The great advantage of the method of Woodiwiss is its simplicity, it does not require identification of species of benthic animals.

However, when using this method, one should remember that under the conditions of sparse fauna, especially on pure sands, more samples must be collected for more correct evaluation. Otherwise, unjustifiably low values of the biotic index may be obtained.

The method of Woodiwiss has been used on some English and French rivers. Investigations associated with this study have shown that it may be used on the water-bodies of the West, North-West and Center of the European USSR. For wider applicability over all of the USSR, it is necessary to perform special investigations of the fauna of different area. In this way the peculiarities of the fauna in various zoogeographical regions will be considered.

Recently, indices of species diversity are used to evaluate the degree of water pollution. Among the most frequently used is the Shannon calculation of the Wilm and Dorris index. This index was applied to the present study on samples of phytoplankton, zooplankton, zoobenthos as a group, and separately for the chironomid larvae. It was found that the values calculated by this index are not solely a function of the degree of pollution. The diversity index calculated by the composition of zoobenthic samples exposed to similar levels of contaminants had lower values on stations with uniform habitats. Further, when species of large size ranges prevail, the index also becomes slower. Considerable seasonal variation also occurs at a given station. The hatch of aquatic insects and departure of the imago stage will have a substantial impact on the seasonal dynamics of the zoobenthic community. Pollution is only

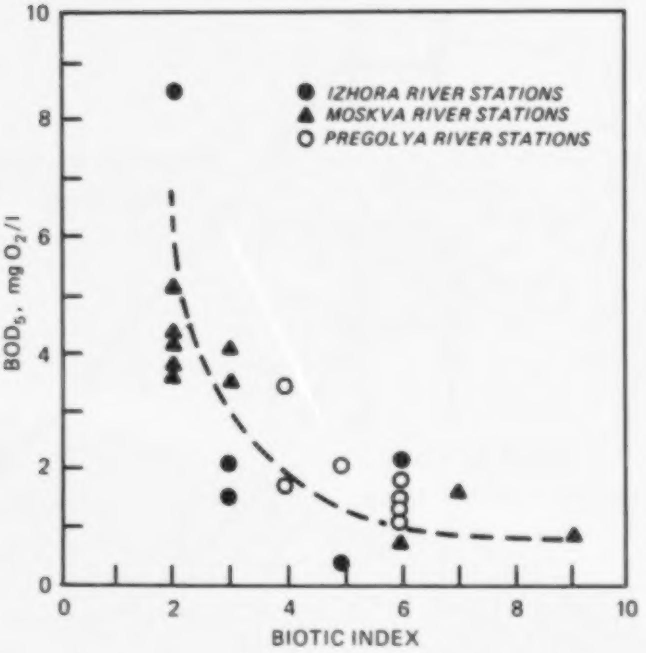

Figure 1. Correlation between Woodiwiss' biotic index and BOD_5.

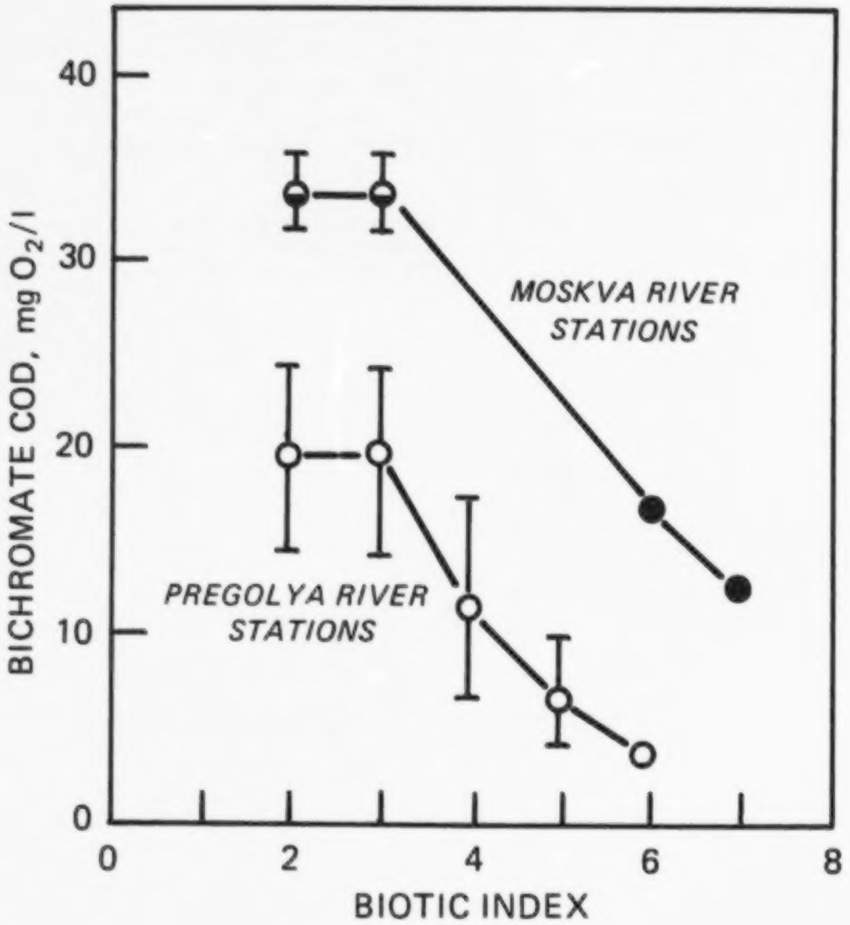

Figure 2. Correlation between Woodiwiss' biotic index and bichromate oxygen consumption.

one of the possible causes of a decrease in the species diversity index. Thus, this methodology may only be used in conjunction with other tecniques as one of the comparative methods.

Analysis of the data obtained in this study enabled the development of a new methodology for assessing the level of water pollution.

In various systems advocating the use of indicator organisms, attention is usually directed to the macrobenthos, ignoring the organisms of meiobenthos. However, meiobenthic organisms may serve as good indicators of the degree of water pollution. The investigations of the composition of meiobenthos performed by S.Ya. Tsckholikhin as a part of this study on various parts of the Moskva River have shown that the representatives of two subclasses of nematods (Adenophorea and Secernantea) may be successfully used as indicators of pollution. The subclass Secernantea tend to occur in places containing large quantities of organic matter. Adenophorea, however, prefer unpolluted waters. Ratios of the numbers of the representatives of these subclasses may serve as an index of the presence and degree of pollution. It is apparently sufficient to identify these organisms to the order classification. This, of course, presents no serious difficulties. Further, as a result of their world-wide distribution, no geographical restrictions are imposed for using nematods as indicators of pollution.

In the lists of indicator organisms proposed by different investigators, the number of the chironomid larvae does not exceed 10, and most frequent used are the larval forms identified to genus. Some representatives of the family Chironomidae are considered to be most numerous in polluted water, e.g., *Chironomus*, *Procladius*, *Psectrotantpus*. Further definition of the use of species of chironomid larvae as indicators of water pollution is presently impossible because of the lack of taxonomic detail for this group and the need for further understanding of the ecological requirements for separate species of this group.

It does appear, however, that the use of universally occurring chironomid larvae holds real potential for hydrobiological analysis. Investigations by E.V. Balushkina as a part of this study have shown that a regular change in the ratio of chironomid larvae belonging to the subfamilies Chironomidae, Tanypodinae, Orthocladiinae takes place in polluted waters. Clean waters are dominated by Orthocladiinae larvae, and polluted waters by Tanypodinae larvae. A pollution index (K) may be developed based upon the relationship between the representatives of these three subfamilies:

$$K = \frac{\alpha_t + 0.5\ \alpha_{ch}}{\alpha_{or}}$$

where:

α_t = index value of Tanypodinae
α_{ch} = index value of Chironomidae
K_{or} = index value of Orthocladiinae

The value α = N+10, where N is relative abundance of individuals of each of the subfamilies in percent of the total abundance of the chironomid larvae. The value 10 is introduced to set limits for changes in the value of the index, K. For example, an increase of this number leads to decrease in the range of possible values of K, and simultaneously to decrease in its sensitivity. At 10, an optimum relation of the gradation of the index and its sensitivity is attained. Since in clean waters the relative abundance of the Orthocladinae larvae is close to 100 percent, and in the most polluted waters the abundance of Tanypodinae larvae approaches 100 percent and the larvae of the subfamily Chironomidae inhabit both clean and polluted waters, the indicator value of chironomids (α_{ch}) for the evaluation of the index K is reduced to one half.

Possible changes in the value of this index in natural waters lie within the limits of 0.09 and 21. Determination of the value of this index of subfamily composition of the chironomid fauna in the rivers studied in this investigation, and in other reports (Gromov, 1950), have shown regular increases with water pollution. In the cleanest waters, K values varied from 0.136 to 1.08, and in the most polluted, from 0.9 to 11.5. Identification of chironomid larvae to subfamily is not difficult. Estimation of the index value K is relatively simple, and it appears to accurately reflect the degree of pollution of a river.

A critical review of methods using oligochaets for evaluation of water quality has shown that the most suitable index is that of Goodnight and Whitley (1961). In the opinion of N.P. Finogenova and A.F. Alimov, an index characterizing the role of oligochaets in the total biomass, but not in the total abundance of animals may be developed in additon to Goodnight and Whitley's. The value of this index increases with an increase in pollution.

The littoral zooplankton community of polluted waters is characterized by a decrease in the total number of crustaceans with an increase in pollution. Simultaneously, as has been shown by M.B. Ivanova in this study, a regular decrease in species composition and abundance of cladocerans occurs, and copepods dominate over cladocerans. In the most polluted areas, the crustacean zooplankton is represented only by cyclopoids. The least sensitive to pollution appears to be *Eucyclops serrulatus*.

All the indices suggested by this study have the distinct advantage of less rigorous taxonomic requirements. These indices also use broader taxomic categories, which naturally increases their wider applicability. It is probable, however, that evaluation of the degree of pollution can not be based solely on these indices. They should be considered as supplemental, since the validity of each may be different in various situations.

Further investigations are required to make the methods of hydrobiological analysis more exact, to determine the most substantial systems of analysis, and to clarify both the lists and indicator value of separate species under various conditions, and in various geographical regions.

It is impossible to develop any system of hydrobiological analysis suitable for all conditions and all communities of aquatic organisms. When working with different communities, under different conditions, and with different purposes, it is necessary to use different methods, choosing the most suitable for a given case.

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SECTION 15

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF SESTON AS INDICES OF WATER POLLUTION

A.P. Ostapenya

Plankton has long been a traditional part of ecological investigations related to water pollution. Accumulated data show convincingly that seston, including planktonic, detrital and mineral suspensions are an important, distinct structural component of aquatic ecosystems, functioning as a single entity. Seston actively influences the quality of water. This influence is diverse, and is evident by its action on the production and destruction stages of the biotic circulation.

Structural and functional characteristics of the seston are sufficiently sensitive for use as an indicator in the evaluation of water pollution.

The concentration of suspended substances in unpolluted waters may vary within a rather wide range. A survey of literature values shows that the concentrations of seston in unpolluted lakes, depending on trophic type, varies from 0.1 to 70 mg dry wt/l. In rivers, even greater concentrations of the suspensions may be observed. However, in spite of differences in content of suspended materials, each may be characterized by definite mean concentrations of seston. Since, in general; the influx of nontoxic pollutants causes an increase in the content of suspended substances in water, the zones of pollution within a water-body may be delineated by the increase in concentration of the seston. The River Svisloch serves as a typical example of a polluted stream. As a result of year round observations in 1973, it has been demonstrated that at all stations situated both above and below the source of pollution, no regular seasonal changes in concentration of seston are observed. This apparently suggests that autochtonous suspension plays a minimal role, since its concentration is closely associated with seasonal changes in the production processes. On the cleaner parts of the river, the concentration of seston varied from 7 to 25 mg/l. Below the outfall, the concentration was greatly increased. Further downstream, approximately 50 kilometers, the content of the seston in the water continued to increase, apparently at the expense of heterotrophic synthesis. During all seasons, seston concentrations were approximately three times greater than the unpolluted sections of the river. The distribution of seston at various stations on the River Svisloch during August of 1973 is shown in Figure 1.

The extent of the polluted zone is evident from the concentrations of suspended material in the river. Approximately 160 km below the source, seston values again achieve levels characteristic of non-polluted portions of the river.

Depending on the intensity of the production processes and the presence of nontoxic substances in waters, the correlation between dissolved and suspended organic matter varies markedly. Usually, dissolved organic substances in unpolluted waters greatly exceed suspended materials. The processes of eutrophication and pollution of waters leads to a considerable increase in suspended organic matter. Thus, in mesotrophic water bodies, suspended organic matter constitutes about 10 percent of dissolved materials, while in eutrophic waters, the relative content of suspended matter reaches 80 percent or higher. In heavily polluted streams, the content of suspended substances may greatly exceed the content of dissolved organic matter.

In the unpolluted part of the Svisloch River, the seston makes up 39 percent of the dissolved organic matter. In a significant part of the river below the source of pollution, the relative content of seston rises to 14 percent of dissolved organic matter (DOM). As a result of self-purification processes, within 60 m, the relative content of the seston drops to 17 percent. Thus, the correlation between dissolved and suspended organic matter may serve as a convenient indication of the zones of eutrophication and pollution.

Relative chlorophyll content in seston is also a rather sensitive index of pollution, and may be used for evaluation of the influence of pollution on aquatic ecosystems. Generally, the relative chlorophyll content in the seston of the polluted zone is higher than in a clean part of the river. Table 1 shows data on the relative chlorophyll content in the seston of three rivers in Belorussia. All the three streams are moderately polluted by domestic and industrial wastes. The relative chlorophyll content in the seston below the source of pollution increased in the Pripyat, Western Dvina, and Neman by 25, 100, and 70 percent, respectively.

Under the stress of heavy pollution by waste waters, the relative chlorophyll content in the seston may notably decline as a result of large quantity of allochtonous suspensions. Data on the relative chlorophyll

TABLE 1. RELATIVE CHLOROPHYLL CONTENT OF SESTON IN CLEAN AND POLLUTED PARTS OF RIVERS

River	Chlorophyll in Seston, %	
	Clean Part	Polluted Part
Pripyat	0.040	0.050
Western Dvina	0.052	0.108
Neman	0.224	0.390

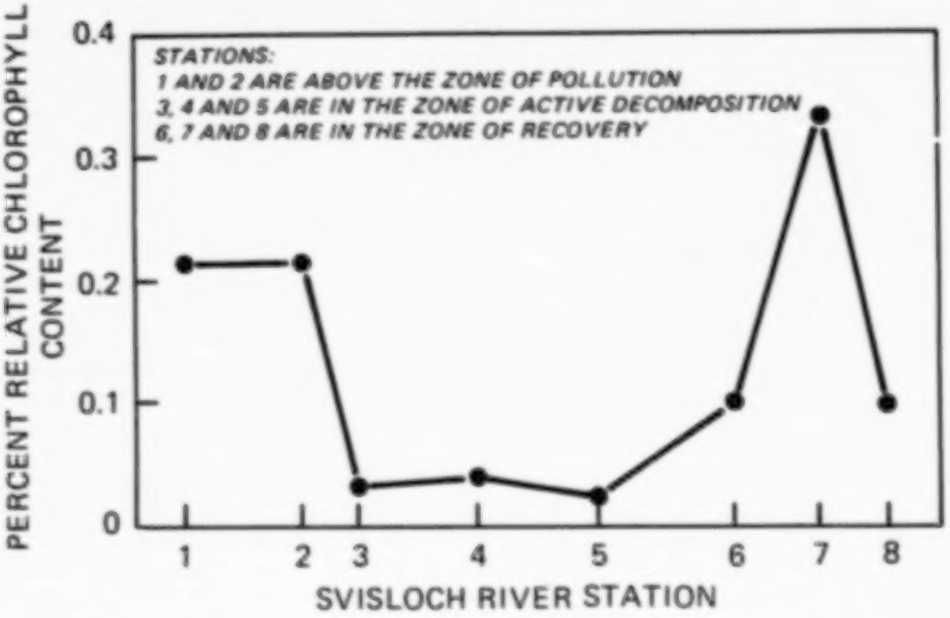

Figure 1. Distribution of seston in the River Svisloch in August of 1973.

content in the Svisloch River are given in Figure 2. Above the source of pollution (Stations 1 and 2), the fraction of chlorophyll in the seston made up 0.2 percent. In the polluted zone (Stations 3, 4, and 5), it dropped to hundredths of a percent. Downstream, the processes of self-purification lead to a considerable increase in the chlorophyll content in the seston.

Specific Oxygen Consumption (SOC) is an important functional index, characterizing the biological activity of seston. It represents the amount of oxygen consumed by a unit mass of suspension, per unit time. According to Hargrave (1972) SOC values for suspensions of various origin, composition and degree of dispersion lie within a range of 0.002-0.240 mg O_2/mg organic matter per day. While the relative values of SOC are apparent, for many scientific and practical purposes, including the evaluation of water pollution, it is necessary to fully understand how this index depends on the trophic status and the pollution degree. In this regard, a determination of SOC by seston has been made on lakes of various types polluted to varying extent by domestic and industrial sewage.

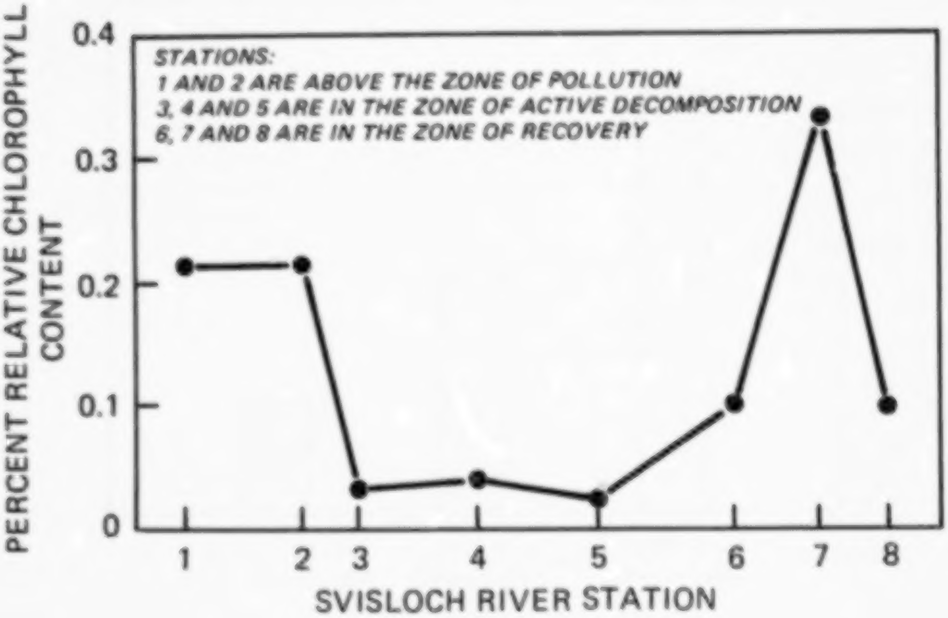

Figure 2. Relative chlorophyll content in seston of the River Svisloch in June of 1973.

The mean SOC values in three lakes are given in Figure 3. In the mesotrophic waters of Lakes Naroch, the seston SOC was the highest averaging 0.062 mg O_2/mg organic matter per day for the vegetation season. With an increase in the trophic status, the SOC notably decreases, reaching a level of 0.036 mg O_2/mg/day in eutrophic Lake Myastro, and 0.016 mg O_2/mg/day in ultra-eutrophic Lake Batorin.

The SOC of seston with a greater fraction of allochtonous organic matter as a result of sewage pollution is demonstrated by studies of the Svisloch River. In clean waters of this river, the SOC made up 0.08 mg O_2/mg of organic matter per day. In polluted portions, the value obtained was 0.147 mg O_2/mg of organic matter per day. A similar SOC value (0.13 mg O_2/mg/day was observed for a polluted part of the Dnieper River.

In general, the data suggests that concentrations of SOC in unpolluted waters range from 0.01 - 0.10 mg O_2/mg of organic matter per day. In the presence of easily degradable allochtonous materials, the SOC values asso-

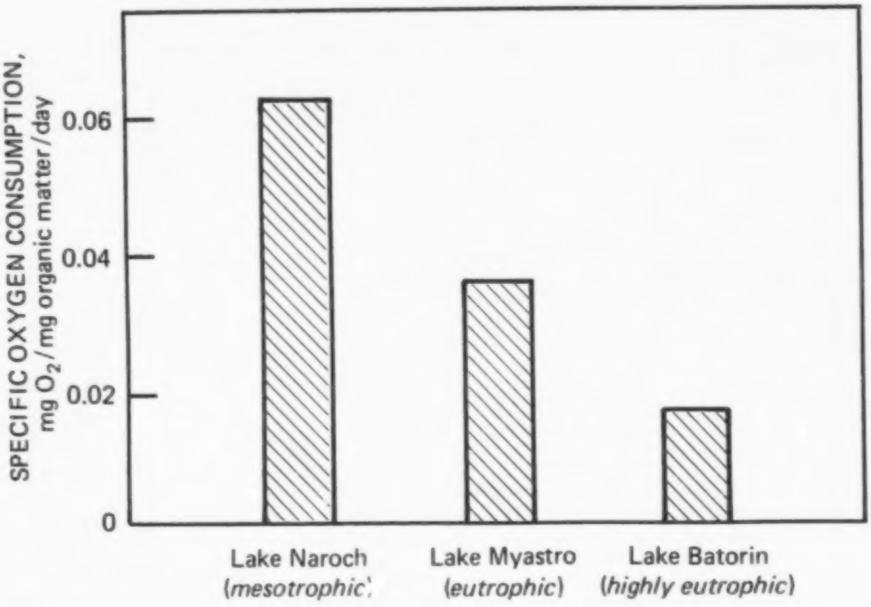

Figure 3. Specific Oxygen Consumption (SOC) by seston in three representative lakes.

ciated with seston increase to 0.15 mg O_2/mg of oganic matter/day. It appears that the metabolic activity of river seston is higher than that of the lakes.

The photosynthetic activity of phytoplankton is an important component ofthe living fraction of seston. It depends on the quality of water, and may be used as an index of pollution. Under the influence of moderatepollution by nontoxic wastes, photosynthesis in the zone of pollution rises, apparently at the expense of enrichment of the water in nutrients. The Neman River graphically demonstrates this reaction in response to an influx of sewage (Figure 4). Below the inflow, photosynthesis increased by three fold. Depending on the intensity and character of pollution, photosynthesis either increase or decrease markedly. In all cases, a notable deviation of photosynthetic activity from the average level characteristic of clean waters is observed in the polluted zone. it should also be noted that such generally accepted indices as oxygen consumption is more highly variable than photosynthetic activity, and yields less precise results when pollution levels are low.

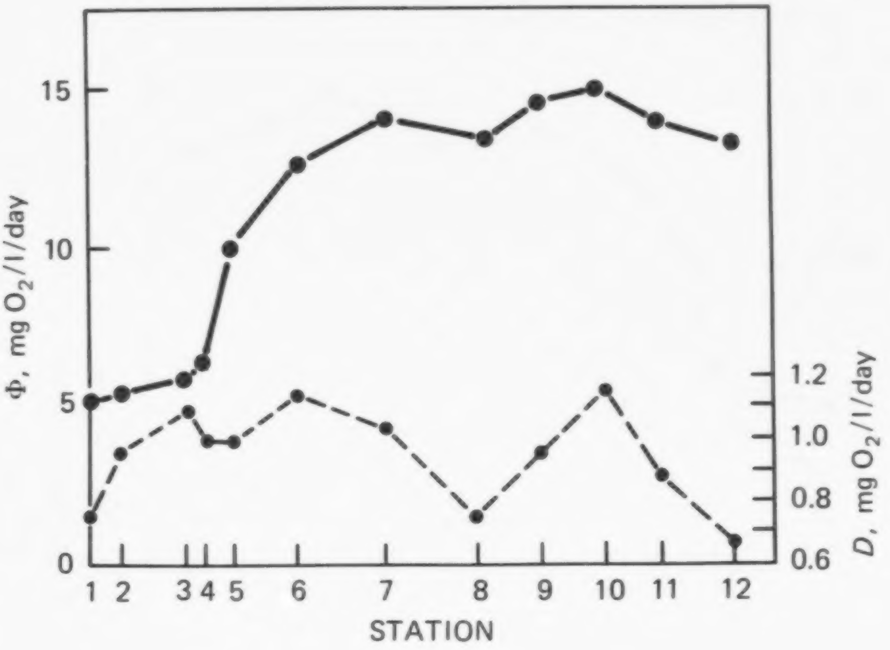

Figure 4. Relationship of photosynthesis (Φ) and distruction (D) in the Neman River in August of 1975.

In the past, investigators have used the ratio of phytoplankton photosynthesis to oxygen consumption by seston and by dissolved organic matter (Φ/D). This ratio is also a rather variable index, and apparently, can not be used for the evaluation of pollution. Much better results are obtained by the use of the ratio Φ/R, where R is total consumption of oxygen by the water and sediments. Figure 5, the ratio Φ/D and Φ/R for the Pripyat River are compared. The ratio Φ/R serves as a better indicator of pollution than the ratio of Φ/D (Figure 5, Stations 1 and 7).

Thus, the structural peculiarities and functional indices of seston reflect the biological activity of suspended substances, and react measurably to pollution. As a result, they may be used for evaluation of the degree of pollution and estimation of water quality. However, because of the diversity of pollutant types and variations between water masses, some variation in measured values can be expected. In some instances, an increase in the index value is observed, and in others a decrease is noted. It is important, however, that in all the cases, a notable deviation from the average clean water statistical norm is observed.

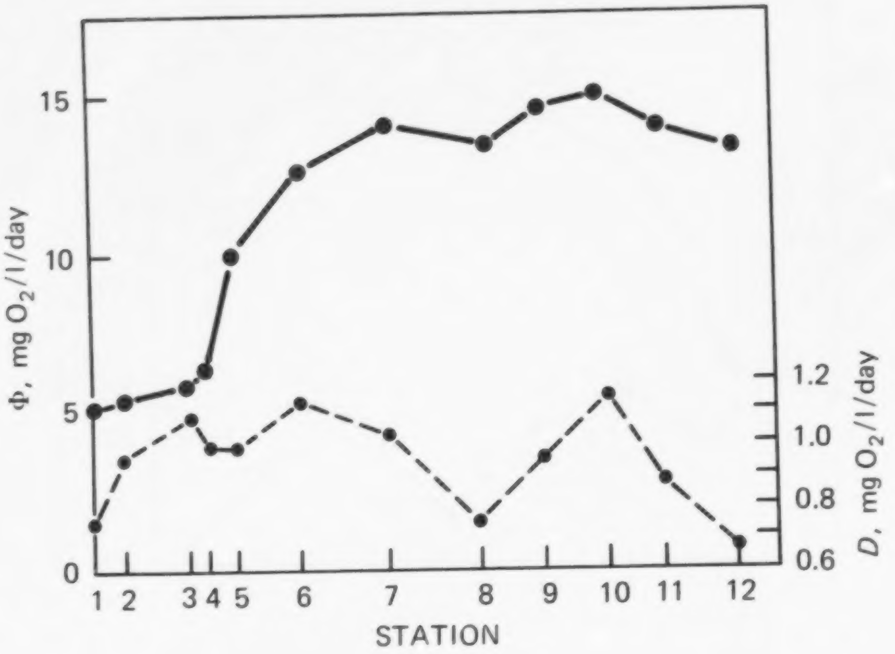

Figure 5. The ratio of Φ/D and Φ/R on the Pripyat River.

The use of structural and functional indices of seston for evaluation of water quality holds promise. Many parameters characterizing suspended matter and its participation in the biotic circulation can be determined by present instrumentation and, thus, even finally may be incorporated in programs of automatic sampling of the control of water quality.